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Short-Chain Fatty Acids Enhance Intestinal Adaptation in Rats Receiving Total Parenteral Nutrition: A Multiorgan Analysis

by

Kelly Anne Tappenden ©

A thesis submitted to the Faculty of Graduate Studies and Research
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In

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled The Multiorgan Response to Short-Chain Fatty Acid Supplemented Total Parenteral Nutrition in Rats submitted by Kelly Anne Tappenden in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Nutrition and Metabolism.

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To my husband, Trevor -

For all the joy you've brought to my life.

ABSTRACT

Following massive small bowel resection, the remnant intestine undergoes morphological and functional adaptive changes. Prior to these adaptive events, short bowel syndrome is characterized by diarrhea, weight loss, electrolyte abnormalities and multiple nutritional deficiencies. Accelerated skeletal muscle proteolysis and translocation of amino acids from skeletal muscle to visceral organs follows surgery. In addition, surgery has immunosuppressive effects by decreasing lymphocyte populations, granulocyte function, NK cell cytotoxicity and delayed hypersensitivity skin-test response. Current parenteral formulations do not provide nutrients specific to the gastrointestinal tract and result in gastrointestinal atrophy. To determine if SCFA supplementation of TPN influences the multiorgan response to surgery, male Sprague-Dawley rats underwent an 80% proximal jejunoileal resection and jugular catheterization. Animals were randomly assigned to receive standard total parenteral nutrition (TPN) or an isoenergetic, isonitrogenous TPN supplemented with SCFAs. SCFA-supplemented TPN reduced loss of skeletal muscle mass by 7 days following surgery which was further supported by decreased urinary 3-methylhistidine excretion, reduced epitrochlearis muscle protein degradation rate and higher plasma glutamine concentration. SCFAs enhanced various components of natural immunity following intestinal resection. SCFA-supplemented TPN prevented the abnormal hematological profiles exhibited in the standard TPN groups and enhanced the cytotoxic activity of natural killer cells. SCFA-supplemented TPN enhanced morphological and function adaptation of the remnant ileum. Abundance of proglucagon and ornithine decarboxylase mRNA were higher in the SCFA groups suggesting that these genes may be involved in SCFA-induced intestinal adaptation. To further examine mediators involved in the enterotrophic response to SCFAs, SCFA-supplemented TPN was infused for 6 to 72h in rats with normal, unresected small intestine. Jejunal mRNA abundance of the basolateral glucose transporter, GLUT2, was increased as early as 6 hours following SCFA-supplementation. Systemic administration of SCFAs resulted in the rapid upregulation of proliferative genes within the ileum such proglucagon, c-*myc*, c-*jun*, and c-*fos*. In conclusion, systemic administration of SCFAs benefits multiple organ systems following surgical trauma. The observation that enterotrophic genes are acutely upregulated following SCFA administration suggests a potential mechanism whereby SCFAs modulate intestinal adaptation. With this understanding, nutritionists will be able to devise new strategies to manipulate the intestine to maximize nutrient utilization.

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LIST OF ABBREVIATIONS

3-MH	3-methylhistidine
ANOVA	analysis of variance
CCK	cholecystokinin
cAMP	cyclic adenosine monophosphate
cDNA	complimentary deoxyribonucleic acid
ConA	concanavalin A
DFMO	α-difluoromethyl ornithine
DNA	deoxyribonucleic acid
EGF	epidermal growth factor
GLP-1	glucagon-like peptide-1
GLP-2	glucagon-like peptide-2
GLUT2	sodium-independent glucose transporter
GLUT5	sodium-independent fructose transporter
Ig	immunoglobulin
IGF	insulin-like growth factor
IGF-1	insulin-like growth factor-1
IGF-2	insulin-like growth factor-2
IGFBP-3	insulin-like growth factor binding protein-3
IL-1β	interleukin-1β
Iono	ionomycin
K_M	Michaelis constant
KRB	Kreb's-Ringer bicarbonate
KRH	Krebs-Ringer HEPES
LU	lytic units
mRNA	messenger ribonucleic acid
MSBR	massive small bowel resection
Na^+, K^+-ATPase	sodium, potassium-adenosine triphosphatase
NK cell	natural killer cell

ODC	ornithine decarboxylase
PMA	phorbol myristate acetate
PYY	peptide YY
RNA	ribonucleic acid
SCFA	short chain fatty acid
SEM	standard error of the mean
SGLT-1	sodium-dependent D-glucose cotransporter
SI	stimulation indices
sIgA	secretory immunoglobulin A
TNF	tumour necrosis factor
TPN	total parenteral nutrition
V_{MAX}	maximal transport rate
WBC	white blood cells

CHAPTER 1

LITERATURE REVIEW

OVERVIEW

Massive small bowel resection (MSBR) results in "short bowel syndrome" which is clinically characterized by diarrhea, weight loss, electrolyte abnormalities and multiple nutritional deficiencies. Advances with home total parenteral nutrition programs have provided many patients suffering from short bowel syndrome with nutritional support. However, information regarding the physiology of intestinal adaptation could lead to therapeutic refinement.

Resection of a portion of the small intestine is a widely used model for studying the process of intestinal adaptation whereby the gastrointestinal tract responds to variations in its environment. Surgical manipulations led Senn to discover the phenomena of intestinal adaptation over 100 years ago (Senn, 1888). Since that time, efforts have focused on morphological and functional aspects, underlying mechanisms, and most recently intracellular and molecular events occurring parallel to the adaptive response. The purpose of this review is to consider the multiorgan response to surgical stress, the morphological and functional reactions to intestinal insult, and to explore possible mechanisms of intestinal adaptation involving various nutritional, humoral and cellular mediators.

THE MULTIORGAN RESPONSE TO SURGICAL STRESS

The physiological and metabolic response to surgery is predictable and well described. These responses include altered protein homeostasis (Cuthbertson, 1930), hypermetabolism (Kinney, 1980), modified carbohydrate metabolism (Imamura et al., 1975), sodium and water retention (Lequesne et al., 1985), and increased lipolysis (Meguid et al., 1974). These responses are mediated through complex interactions between many organ systems including the immune, skeletal muscle and gastrointestinal systems.

Surgical trauma results in significant abnormalities of cellular immunity (Tellado-Rodriguez and Christou, 1988) including delayed skin test response (Harvey et al., 1981; Johnson et al., 1979), impaired natural killer (NK) cell cytotoxic activity (Pollock et al., 1991) and increased suppressor T-cell abundance (Wood et al., 1984). During the first 7-10 days following surgery, increased prostaglandin E_2 production inhibits cytokine secretion (Faist et al., 1988; Wood et al., 1984), which are believed to mediate multiple aspects of the stress response.

Alterations in protein and amino acid metabolism following surgery result in a net loss of lean body mass. Whole-body nitrogen loss increases, primarily due to skeletal muscle protein degradation (Cuthbertson, 1931). The pattern of intracellular amino acids in skeletal muscle, the largest pool of free amino acids in the body (Bergström et al., 1974), undergoes characteristic changes. Glutamine and basic amino acid concentrations decrease, whereas branched-chain and aromatic amino acid concentrations increase (Vinnars et al., 1975; Askanazi et al., 1980). By the third day following surgery, intracellular muscle glutamine concentration is markedly depressed (Roth et al., 1985), yet it remains unclear if low intracellular glutamine alters protein turnover rate (Fang et al., 1995). These alterations in skeletal muscle intracellular amino acids arise from a translocation of amino acids from skeletal muscle to visceral organs. Glutamine and alanine account for 60% of the amino acid nitrogen released by muscle during stress states, an event which is mediated by glucocorticoid hormones (Souba et al., 1990b). Despite accelerated skeletal muscle release during critical illness, circulating concentrations of glutamine are diminished, which indicates that accelerated uptake occurs in other tissues. The small intestine is the principal organ of glutamine uptake and metabolism in the body in both the normal and stress states (Souba, 1993).

The gastrointestinal tract has long been viewed as quiescent or inactive during critical illness. However, in addition to the primary role of digestion and absorption of nutrients, the intestine acts as a barrier to intraluminal bacteria, preventing host invasion by micro-organisms or soilage by their toxins. The maintenance of normal epithelial cells prevents transepithelial migration, and the conservation of tight junctions prevents movement via paracellular channels (Marin et al., 1983). In addition to the epithelial barrier, a variety of immunologic mechanisms exist preventing bacterial translocation. Together with lymphocytes, macrophages, and Peyer's patches, the regional lymph nodes provide an extensive primary host immune defence system. The intraluminal secretion of secretory immunoglobulin A (sIgA) provides a specialized defence system and aids in recognition of appropriate antigens for gastrointestinal absorption while preventing enteroadherance (Burke et al., 1989). Finally, a backup barrier is supplied by the Kupffer cells of the liver and the spleen which trap and detoxify bacteria and their products should they penetrate beyond the epithelium and the regional lymphatic tissue. An intact epithelial barrier and a normal functioning immune system are both important for adequate barrier function. However, during states of immunosuppression, the intact mucosa functions solely to provide an effective barrier, suggesting that cell-mediated immunity serves a secondary or supportive role to the epithelium (Maddaus et al., 1986).

INTESTINAL ADAPTATION FOLLOWING SURGICAL RESECTION

Proliferative and Morphological Response During Intestinal Adaptation

Following MSBR, investigators have noted dilation, lengthening, and/or thickening of the remnant intestine (Wilmore et al., 1971; Sigalet et al., 1990). This morphological adaptation can be attributed primarily to a hyperplastic response (Dowling and Booth, 1967). The remnant segment exhibits an expansion of the proliferative compartment of the crypts in the postanastomotic area (Hanson and Osborne, 1971; McDermott and Roudnew, 1976), a reduction of the cell cycle time (Hanson and Osborne, 1971; McDermott and Roudnew, 1976; Loran and Crocker, 1963), and an increased number of cells migrating more rapidly from the crypts to the villi (Hanson, 1982). The resulting effects are increases in mucosal cell mass, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein contents (Williamson, 1978). The magnitude of the adaptive response after MSBR is greater after proximal as opposed to distal resections (Williamson, 1978), is maximal immediately downstream of the anastomosis (Williamson, 1982a), and is proportional to the amount of small intestine resected (Hanson et al., 1977a).

The hyperplastic response to MSBR leads to an increased number of epithelial cells per unit length of crypt/villus (Hanson et al., 1977a). This enhanced population of cells occurs secondary to increased cell turnover in the proliferative zone of the crypts (Sagor et al., 1982; Sagor et al., 1983). Individual enterocytes thus engendered spend less time in the differentiation zone of the crypt (Menge et al., 1982), and have been considered to be functionally immature (Menge and Robinson, 1978; Morita et al., 1982). The mechanisms governing both the proliferative and functional changes following MSBR will be discussed in a later section.

Functional Response During Intestinal Adaptation

Following intestinal resection, there is evidence that the hyperproliferative state is accompanied by various functional modifications. Functional parameters noted to be altered include the digestion and absorption of nutrients due to variation in mucosal enzyme activities (Weiser, 1973) and the function of brush border membrane transport proteins (Garvey et al., 1976; King et al., 1981; Kinter and Wilson, 1975).

Following a proximal small bowel resection, the hyperplastic remnant exhibits changes in the activity of various mucosal enzymes. When related to unit length of intestine the adapting intestinal remnant displays increased activities of numerous enzymes (Bochanek et al., 1973; Bury, 1972; Gleeson et al., 1972a; Urban, 1977; Urban and Haley, 1978; Urban et al., 1982). However, these activities are either unchanged or reduced, when expressed in terms of the protein or DNA content of the mucosa (Feldman et al., 1976; Gleeson et al., 1972a, b; McCarthy and Kim, 1973). Thus, alterations in enzyme activities appear to be involved in the process of intestinal adaptation, however it is difficult to interpret the influence of intestinal resection on the specific activity of mucosal enzymes.

MSBR has been shown to alter enterocyte absorption of various nutrients. Adaptative increases of sugar and amino acid uptake (Gleeson et al., 1972b; Robinson et al., 1982; Thomson, 1986) is thought to result from alterations in the maximal transport rate (V_{MAX}) as opposed to a change in the Michaelis constant (K_M) or a generalized change in sodium gradients or intestinal morphology (Karasov and Diamond, 1987). The passive uptake of lipids responds heterogeneously to MSBR, as the uptake of medium-chain but not long-chain fatty acids, or saturated but not unsaturated long-chain fatty acids are altered (Thomson et al., 1986). This kinetic data suggests that through an increased number and perhaps various qualitative changes of transport proteins, the process of intestinal adaptation results in the absorption of selected nutrients aimed at meeting altered nutritional requirements.

The altered nutrient transport that follows MSBR may be due to changes in cell kinetics and villus transit time. Various researchers have suggested that the increased cell turnover following MSBR leads to a stimulation of cell migration rate along the villus, resulting in a reduced life span of the individual cells (Menge and Robinson, 1978). The subsequent reduction in the life span is not thought to be adequate for enterocyte differentiation thus rendering a single enterocyte underdeveloped and functionally inept (Menge and Robinson, 1978; Smith, 1984; Smith, 1986). Furthermore, in the latter stages of enterocyte differentiation, the rate at which absorption increases is doubled (Smith, 1986). This alteration in the distribution of transporting enterocytes along the villus suggests that enterocyte differentiation is controlled in part by a positional signal (i.e. the crypt/villus junction) rather than one based exclusively on enterocyte age (Cheeseman, 1986; King et al., 1983).

With the recent cloning and sequencing of various nutrient transporters, it is now possible to characterize functional adaptations at the molecular level (Figure 1.1). The Na^+-dependent glucose co-transporter (SGLT-1) transports glucose and galactose from the lumen across the brush-border membrane (Semenza et al., 1984; Stevens et al., 1984; Wright, 1993). Energy for this step is obtained by coupling hexose transport to sodium and electrochemical gradients. The sodium gradient is maintained by the basolateral Na^+, K^+-ATPase which actively transports sodium out of the enterocyte (Crane et al., 1961; Wright et al., 1994). Fructose is transported across the brush border membrane by the sodium-independent facilitative transporter, GLUT5 (Burant et al., 1992a, b; Crouzoulon and Korieh, 1991; Davidson et al., 1992; Mahraoui et al., 1992; Rand et al., 1993a, b), which has recently been localized to the jejunal basolateral membrane in humans (Blakemore et al., 1995). GLUT2, the basolateral facilitative transporter, shuttles all 3 hexoses between the enterocyte and systemic circulation (Thorens et al., 1988, 1990; Burant and Bell, 1992; Cheeseman, 1993; Colville et al., 1993; Maenz and Cheeseman, 1987). It is well established that dietary substrate can modulate the levels and activity of their respective transporters (Ferraris and

Diamond, 1989; Shirazi-Beechey et al., 1995). A high-carbohydrate or high-protein diet stimulates intestinal D-glucose or amino acid transport, respectively, within 24 hours of consumption (Ferraris and Diamond, 1993). SGLT-1 expression dramatically increased in the ileum of rats, within 6 hours of a 70% intestinal resection (Hines et al., 1994). The effect of intestinal resection on other nutrient transporters awaits further study.

Although the absorption of various nutrients by the post-resection enterocyte is altered, it is difficult to interpret the current pool of data as the results conflict in response to variation in the denominators used to express this data. For example, *in vivo* studies of the amino acid transport properties of the intestinal remnant have revealed that the absorptive capacity related to intestinal length increases, while that related to mucosal DNA content decreases (Thomson et al., 1989). Thus, the absorption of nutrients is modified following MSBR; however, it remains difficult to resolve the extent and direction as the alteration depends on the method of expressing the results.

A large pool of data exists indicating that MSBR leads to alterations in the remnant segment, however there is a dissociation between the structural and functional changes following resection (Urban et al., 1982; Urban and Michel, 1983; O'Loughlin et al., 1994). The increase in *in vivo* glucose and amino acid absorption per unit intestine length is due to increased mucosal mass rather than absorptive function per enterocyte and lags behind structural adaptation (Weser and Hernandez, 1971; Feldman et al., 1976; Urban and Michel, 1983; Garrido et al., 1979). This evidence suggests that the functional adaptation in the brush border membrane is achieved independently of the morphological adaptation, thus raising the possibility that separate signals designate the various morphological, digestive, and transport changes involved in the process of intestinal adaptation.

EFFECT OF NUTRIENTS ON INTESTINAL ADAPTATION

The important role of nutrients in small intestinal mucosal regeneration has long been recognized. For example, during total starvation or severe protein-energy malnutrition, the enteric mucosal and muscular layers atrophy to a disproportionate degree compared to the changes in total body mass and weight of other tissues (Steiner et al., 1968; McManus and Isselbacher, 1970). The route and type of feeding also influences intestinal cell proliferation (Levine et al., 1974; Maxton et al., 1987; Ford et al., 1984; Hosada et al., 1989), indicating that nutrition plays a pivotal role within the complex host of factors that may be involved in the process of intestinal adaptation.

Total Parenteral Nutrition

Total parenteral nutrition (TPN) is a means of providing protein, carbohydrate, fat, vitamins, and minerals to those persons who are unable to assimilate nutrients provided via the gastrointestinal tract. Following MSBR, TPN is frequently administered to prevent dehydration and malnutrition. If intestinal adaptation is insufficient, partial or total dependence on parenteral nutrition may be required to support the patient, sometimes permanently. Although TPN has proved to be life-sustaining for many patients, this mode of therapy is frequently associated with debilitating complications (catheter sepsis, micronutrient deficiencies, bone demineralization; Burnes et al., 1992; Leaseburge et al.,. 1992; Koo, 1992), as well was significant costs estimated to be $75,000 - 150,000 US per patient year (Howard et al., 1991). When coupled with research suggesting that the use of standard TPN inhibits the process of intestinal adaptation following MSBR (Koruda et al., 1988b), one could speculate that use of this necessary form of nutritional support in the transitionary period prior to intestinal adaptation may be detrimental to the adaptive response and confine its users to long-term use for maintenance of an adequate nutritional status.

Morphological and proliferative changes occur in the gastrointestinal tract of animals nourished totally by parenteral feeding (Johnson et al., 1975; Levine et al., 1974; Hosada et al., 1989). Johnson and associates (1975) demonstrated that rats maintained on intravenous feeding for up to 3 weeks exhibited significant decrease in weights of the oxyntic gland area of the stomach, small intestine, and pancreas compared to controls fed an isocaloric oral diet. The small intestine of the parenterally fed animals exhibited significant loss of DNA, and a near doubling of the RNA:DNA ratio. More recently, mild villus atrophy and a reduced level of incorporation of ^{3}H-thymidine were observed in intestinal biopsies from children receiving TPN when compared to orally fed children exhibiting symptoms of intestinal disturbances (i.e. abdominal pain or chronic diarrhea; Rossi et al., 1993). This study suggests that the intestinal response to TPN in humans is in general agreement with the hypoplastic effect observed in animals.

The use of TPN has also been shown to inhibit several functional parameters of the small intestine (Levine et al., 1974). Rats receiving TPN showed a marked decrease in brush border enzyme activity, primarily in the proximal small intestine. Human small intestinal biopsies revealed that short-term TPN resulted in reduced activity of lactase, sucrase, and palatinase (Inoue et al., 1993a). In addition, the use of TPN leads to significant changes in the glycoprotein profile in the rat small intestine, suggesting a strong dependency of glycoprotein synthesis on luminal substances (Miura et al., 1992). Recent evidence from human subjects further indicated functional changes following TPN, as shown by a 24-44% decrease in carrier velocity of many substrates including D-glucose, L-alanine, L-arginine, and L-leucine across brush border membrane vesicles (Inoue et al., 1993a).

Although TPN is a popular method of nutritional support for critically ill patients, it fails to support gut mucosal structure or function and has been associated with immunosuppression (Alverdy et al., 1988, 1992; Moore et al., 1989). Marin and associates (1983) suggest that patients may encounter septic episodes by way of transepithelial bacteria migration and movement through paracellular channels due to leaky tight junctions. This altered mucosal permeability may further compromise the intestinal-barrier function and contribute to the chronic hypermetabolism and multiorgan system failure so common to critically ill patients (Wilmore et al., 1988). It is noteworthy that clinical evidence regarding the occurrence of bacterial translocation in humans receiving TPN is lacking (Sedman et al., 1995; Alpers and Stenson, 1996).

In general, when oral or enteric feeding is not possible, the intestinal mucosa can be expected to atrophy. Although mechanistically unclear, this response may be due to incomplete understanding of normal nutritional requirements, the bypassing of important intestinal regulatory mechanisms and/or omission of nutrients and growth factors essential or conditionally essential for the gastrointestinal tract. As the use of TPN in the transitionary period preceding intestinal adaptation is often required to prevent malnutrition secondary to malabsorption, future investigations should focus on the mechanism of intestinal adaptation with the goal of supplementing standard TPN solutions with the factors involved in the hyperplastic response.

Luminal Nutrition

The use of TPN has afforded indefinite survival and consequently enhanced the prognosis that patients with short bowel syndrome would have received only 15 years ago. Goulet and associates (1991) recently reported an increase in survival of children with less than 40-cm of small intestine from 42% before 1980 to 94% in the decade since. However, the successful role of TPN in providing nutritional support for these patients somewhat obscures the reduction in intestinal adaptation that occurs if the intestine is robbed of the stimulus of continued enteral nutrition (Booth, 1994; Johnson et al., 1975a; Wilmore et al., 1988).

A large pool of evidence supports the trophic role of luminal nutrients in the process of intestinal adaptation (Booth, 1994; Dowling, 1974; Morin et al., 1981). Young and Weser (1974) found that rats having undergone a 50% proximal intestinal resection had a much greater degree of hyperplasia in the remnant segment than those having had a 50% distal resection. Much of the evidence supporting the concept that luminal nutrition is trophic to the small bowel mucosa has been appealing in that it is gradient based. The proximal small intestine is routinely exposed to the highest concentrations of nutrients, perhaps explaining the presence of intestinal villus height and crypt depth gradients, the hyperplasia of the distal remnant following a proximal bowel resection, and the observation that the transposition of proximal and distal gradients results in hyperplasia of the former distal segment, and hypoplasia of the proximal segment now located distally (Johnson, 1982). However, one must be cautious in suggesting that the topical exposure to nutrients is exclusively trophic to the small bowel mucosa, without considering the neural stimulation, endogenous secretions, and gastrointestinal hormones whose release these luminal stimuli provoke (Altmann, 1971; Johnson, 1977; Frankel et al., 1994; Thomson et al., 1989; Roberge and Brubaker, 1991).

The specific dietary components of the luminal nutrition supplied have also been shown to alter the morphological and functional characteristics of the adaptive response. Weser and colleagues (1986) demonstrated that disaccharides are more trophic to the rat small bowel than monosaccharides. In addition, high protein diets have been noted to stimulate amino acids transport across the jejunum *in vivo* (Scharrer, 1972) as well as *in vitro* (Lis et al., 1972). Finally, free fatty acids appear to be even more potent than long chain triglycerides, protein, starch or medium chain triglycerides in enhancing many morphological aspects of intestinal adaptation (Morin et al., 1981, Grey et al., 1984). However, amongst long chain fatty acids, eicosapentanoic and docosahexanoic acids are more effective in inducing structural changes associated with adaptation than less highly unsaturated fats (Vanderhoof et al., 1994). The constituents of the diets responsible for enhancing intestinal form and function still require additional research efforts. Once the trophic dietary constituents have been identified, diets can be formulated to optimize the morphological and functional changes associated with intestinal adaptation in patients with short bowel syndrome.

Currently, ample attention is being focused on fuels known to be specific to the intestine - such as glutamine and short-chain fatty acids - and examining the role that they might play in the process of intestinal adaptation.

Glutamine

Glutamine is the most abundant amino acid in the blood and free amino acid pool of the body. In addition to being an important vehicle for the transfer of nitrogen between tissues (Souba, 1987), glutamine is a regulator of protein synthesis (Hammarqvist et al., 1989), the most important substrate for renal ammoniagenesis (Welbourne et al., 1986), and an essential precursor for nucleic acid biosynthesis in all cells (Frisell, 1982). Furthermore, glutamine is a key energy source for rapidly dividing cells such as renal tubular cells, lymphocytes, fibroblasts and epithelial cells of the intestinal mucosa (Windmueller, 1982).

The gastrointestinal tract is the principal organ of glutamine utilization with most of the uptake occurring via sodium-dependent transport in the small intestinal epithelial cells that line the villi (Said et al., 1989). Functionally, glutamine metabolism by the small intestine: a) provides a major source of energy for the gut; b) provides amide nitrogen that may support nucleotide biosynthesis; and, c) processes nitrogen and carbon from other tissues for further metabolism in the liver and kidneys (Souba, 1993).

The enterocytes glutamine as a respiratory fuel and dispose of the majority of glutamine nitrogen as ammonia, alanine, and citrulline. The gastrointestinal tract is well suited to metabolize glutamine since the ammonia produced readily diffuses into the portal blood and is extracted by the liver before reaching the systemic circulation. Other major fuels used by the intestine are the ketone bodies, β-hydroxybutyrate and acetoacetate. Glucose and free fatty acids contribute relatively little energy (Windmueller, 1982).

Researchers have provided convincing evidence that glutamine is important for maintenance of intestinal metabolism, structure, and function. O'Dwyer and collegues (1989) demonstrated the ability of glutamine-supplemented TPN to protect against mucosal atrophy and improve nitrogen retention as compared to standard TPN in rats. The addition of glutamine to TPN resulted in significant increases in mucosal weight, DNA and protein contents, and villus height at an optimal dose of 2 g/100 mL. More recently, Inoue and associates (1993b) reported that following starvation atrophy, rats fed glutamine-supplemented TPN had significant improvement of intestinal mucosal weight, nitrogen content, villus height, mucosal thickness, and mucosal disaccharidase activity as compared to rats fed standard TPN. Similarly, following MSBR, alanyl glutamine-enriched TPN significantly enhanced intestinal adaptation (Tamada et al., 1993). These results reveal that glutamine-supplemented TPN can maintain small bowel mucosa and enhance mucosal cell proliferation following stress, thus preventing the villous atrophy associated with standard hyperalimentation.

The mechanisms by which glutamine exhibits trophic effects on the small intestine are thought to include :

1) a direct effect as a substrate of metabolic pathways of enterocytes by stimulation of trophic factors (i.e. precursors for DNA biosynthesis; Klimberg et al., 1990);
2) an increase in villus height due to augmented cell turnover (Barber et al., 1990; Souba et al., 1990a; Fox et al., 1988);
3) induction of accelerated enterocyte maturation (as measured by high diamine oxidase activity; Luk et al., 1967);
4) stimulation of pancreas secretions (Altmann,1971);

5) increasing protein synthesis in intestinal epithelial cells (Higashiguchi et al., 1993);
6) influencing proliferative hormonal levels (i.e. stimulation of proglucagon-derived peptides, etc.; O'Dwyer et al., 1989).

In addition to maintaining the intestinal barrier to intraluminal bacteria, glutamine-supplemented TPN has been shown to protect against bacterial translocation through immunoenhancement (Castell et al., 1994). Alverdy and associates (1988) demonstrated that standard hyperalimentation results in a significantly lower sIgA concentration, which was positively correlated with bacterial translocation from the gut. On a cellular level, animals maintained on glutamine-supplemented TPN did not differ significantly from chow fed animals. However, the group receiving standard nutrient solutions displayed significant decreases in sIgA and gut lamina propria plasma cells and intraepithelial lymphocytes (Alverdy et al., 1992). Thus, glutamine may be an important nutrient for mucosal immune function during parenteral support.

The effects of glutamine as a conditionally essential nutrient for systemic immune function has received recent attention. *In vitro* studies of peripheral human lymphocytes revealed a correlation between glutamine concentration and lymphocyte proliferation (Parry-Billings et al., 1990). Glutamine also appears to be important for the normal function of phagocytotic cells such as macrophages (Wallace and Keast, 1992) and neutrophils (Ogle et al., 1994). Thus, several studies from various researchers conclude that glutamine is important for several organ systems, suggesting that glutamine is a conditionally essential amino acid during critical illness.

However, due to instability, a short shelf-life and its classification as a non-essential amino acid, glutamine is not included in clinically available TPN solutions, ultimately compromising the nutritional status and prognosis of the critically and chronically ill.

Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are produced in the mammalian intestinal tract as a by-product of anaerobic bacterial metabolism of carbohydrate. Acetate, propionate, and butyrate account for approximately 85% of formed SCFAs and are produced intraluminally in a nearly constant molar ratio of 60:25:15 (Cummings, 1984). Among their various properties, SCFAs are freely absorbed by intestinal mucosa, have a relatively high energy content, are readily metabolized by intestinal epithelium and liver, stimulate sodium and water absorption in the colon, and are trophic to the intestinal mucosa (Rombeau and Kripke, 1990).

Absorption of SCFA varies among locations (Velázquez et al., 1996a), but is believed to occur via three mechanisms: 1) passively in the lipid-soluble undissociated form, likely coupled to a Na^+-H^+ exchanger; 2) a $SCFA^-$- HCO_3^- exchange; and 3) diffusion of the anionic form through paracellular functions (Argenzio and Whipp, 1979; Ruppin et al., 1980; Rechkemmer et al., 1988; Engelhardt, 1995). Once absorbed, the three major SCFA are readily metabolized by colonocytes (Cummings, 1981; Roediger, 1982; Engelhardt and Rechkenmer, 1983; McNeil, 1984; Marty and Vernay, 1984; Awad et al., 1990; Topping et al., 1993). Uptake and metabolism of SCFA is rapid and complete as $^{14}CO_2$ appears in the breath 10 to 15 minutes after administration, and 50% of 1-^{14}C-labelled acetic and propionic acids and 63% of butyric acid appear as $^{14}CO_2$ in the breath within 6 hours (Hoverstad et al., 1982).

Butyrate appears to be the fuel of choice for colonocytes (Desmoulin et al., 1985; Roediger, 1982; Ardawi and Newsholme, 1985; Marsman and McBurney, 1995), as it suppresses glucose oxidation by about 50% (Roediger, 1980). In single substrate systems, about 75% of the oxygen consumed by the colonocytes was attributable to butyrate oxidation (Hoverstad et al., 1982).

The provision of energy results from the production, absorption, and metabolism of SCFAs. It has been estimated by colonic luminal perfusion studies that the human colon has the ability to absorb up to 540 kcal/day in the form of SCFAs (Ruppin et al., 1980). However, in addition to providing energy, SCFA production may influence gastrointestinal function beyond the colon.

There is a growing body of research supporting a trophic role for SCFAs in the small intestine. Intestinal adaptation to resection is increased with the ingestion of fermentable dietary fiber (Koruda et al., 1986; Kripke et al., 1988). Reduction of cecal SCFA content with antibiotic treatment decreases post-resection adaptation (Aghdassi et al., 1994) whereas, intracolonic infusion of SCFAs in germ-free animals stimulates mucosal growth in the small intestine (Sakata, 1987; Frankel et al., 1994). TPN supplemented with SCFAs significantly reduces the ileal mucosa atrophy associated with TPN (Koruda et al., 1990) and enhances adaptive markers following small bowel resection (Koruda et al., 1988b). The provision of triacetin in TPN increases circulating acetate concentrations, improves protein metabolism and reduces intestinal mucosal atrophy in hypermetabolic burned rats (Karlstad et al., 1992). Using *in vivo* models, SCFAs stimulate intestinal epithelial cell proliferation and mucosal growth (Sakata, 1987; Kripke et al., 1989; Frankel et al., 1994), however *in vitro* SCFAs inhibit epithelial cell proliferation (Sakata, 1987; Marsman and McBurney, 1996). The differential response of SCFAs on proliferation *in vivo* and *in vitro* may reflect osmolarity/intracellular pH changes that can't be regulated *in vitro* (Marsman and McBurney, 1996) or a systemic mechanism, which may include neural (Frankel et al., 1994; Sakata, 1976) and hormonal elements (Thompson et al., 1996).

Researchers have speculated on several mechanisms by which SCFAs may influence small intestinal mucosal proliferation. SCFAs, particularly acetate, increase intestinal blood flow which may enhance mucosal proliferation (Dowling, 1982). Intravenous infusion of SCFAs, especially butyrate, increase pancreatic secretions (Harada and Kato, 1983; Al-Mukhtar et al., 1983) and although not extensively studied, are suspected to stimulate enterohormone secretions, such as proglucagon-derived peptides (Koruda et al., 1990;

Thompson et al., 1996; Gee et al., 1996). Apart from the colonic mucosa, the main site of SCFA metabolism is the liver (Remesy et al., 1980). The hepatic metabolism of SCFAs results in the production of the ketone bodies - acetoacetate and β-hydroxybutyrate, as well as the amino acids - glutamine and glutamate (Desmoulin et al., 1985; Cross et al., 1984). Ketone bodies and glutamine are major respiratory fuels of the small intestine. Thus, it appears that via common hepatic metabolism to the preferred intestinal fuel sources, both the portally delivered and intravenously infused SCFAs would have similar effects on the intestinal mucosa.

Current parenteral nutrition formulations do not provide nutrients specific to the gastrointestinal tract. Glutamine is absent from contemporary solutions and ketone generation is suppressed as these solutions contain glucose. In addition, dietary fiber is not presented to the colon thereby suppressing the synthesis of SCFAs by the enteric flora. Thus, one could speculate that current therapy may in effect starve the gut and result in diminished intestinal adaptation and unsatisfactory outcomes for patients with short bowel syndrome.

HUMORAL REGULATION OF INTESTINAL ADAPTATION

Intestinal adaptation is a complex physiological process which is not completely understood. A common mechanism by which the physical presence of food, glutamine and SCFAs have been postulated to influence small intestinal mucosal proliferation is through the stimulation of humoral factors. Isolated loops of bowel normally become hypoplastic. However, following MSBR, this process is reversed and hyperplasia occurs (Hanson et al., 1977b; Williamson and Bauer, 1978; Gornacz et al., 1984). This phenomena argues strongly for the presence of a humoral mitogenic factor. The remainder of this review will concentrate on discussing several of the humoral mediators thought to be involved in intestinal adaptation.

Cholecystokinin

Cholecystokinin (CCK), a hormone produced by the endocrine cells of the duodenum and upper jejunum, is secreted in response to products of fat and protein digestion. As CCK is responsible for increased exocrine secretion of the pancreas and for contraction of the gall bladder, it is difficult to distinguish the possible direct trophic effect of CCK on the gastrointestinal tract from the indirect effect mediated through pancreaticobiliary secretion. Following MSBR, plasma CCK levels rose in parallel with plasma enteroglucagon, increases in crypt cell production rate, and were positively correlated with percentage of intestine resected (Gornacz et al., 1984). Nonetheless, the role of CCK in intestinal adaptation following MSBR is believed to be limited, as infusion of CCK into the intestinal lumen resulted in no alterations in hyperplasia (Dowling, 1982).

Neurotensin

Neurotensin is a tridecapeptide found mainly in the central nervous system and in the mucosal endocrine cells (N cells) in the jejunum and ileum (Helmstaedter et al., 1977). Following MSBR, neurotensin-like immunoreactivity is increased in the jejunum and ileum only in response to intragastric installation of fat (Olsen et al., 1987). When administered subcutaneously into rats fed a liquid elemental diet, neurotensin effectively reversed the associated jejunal mucosal hypoplasia.

Evers and associates (1991) demonstrated a rapid and nutrient-independent increase in neurotensin messenger RNA (mRNA) levels less than 24 hours following MSBR. These researchers suggest that the trophic effects of neurotensin are mediated both indirectly by stimulation of pancreatic juice and directly via a systemic effect on the hypoplastic mucosa (Evers et al., 1989). de Miguel and associates (1994) support this hypothesis by speculating that neurotensin is effective due to it's ability to stimulate the release of enterotrophic proglucagon-derived peptides.

Gastrin

Following MSBR, serum concentrations of gastrin are elevated resulting in acid hypersecretion (Straus et al., 1974; Williams et al., 1985). Gastrin is clearly trophic to the stomach (Johnson, 1987; Johnson et al., 1975b) but mediation of growth beyond the stomach is controversial (Hakanson et al., 1986; Reilly et al., 1995). The adaptive response to refeeding does not correlate with plasma gastrin concentration (Goodlad et al., 1987a, b). Induced endogenous or exogenous hypergastrinaemia in rats has no enterotrophic effects beyond the oxyntic stomach (Hakanson and Sunder, 1991) and jejunum (Mayston and Barrowman, 1973). Thus, while gastrin may be important for gastric adaptation following intestinal resection, it appears to be excluded from the adaptive response of the residual small bowel.

Bombesin

Bombesin, a tridecapeptide which stimulates gastrin release and gastric acid secretion, is also known as gastrin-releasing peptide. Bombesin has been shown to enhance intestinal growth and differentiation in the suckling rat (Lehy et al., 1986). Although bombesin was noted to stimulate proglucagon-derived peptide release, it did not increase cell proliferation following MSBR (Sagor et al., 1985). Bombesin has been shown to increase intestinal secretion of sIgA as well as to augment systemic immunity (Debas and Mulvihill, 1991; Jin et al., 1989). Bombesin may also be important for barrier function in rats as it protects against both diet- and burn-induced bacterial translocation (Yuval et al., 1994; Coffey et al., 1988, Haskel et al., 1994). Bombesin is not thought to have a direct role in intestinal adaptation, however its ability to augment plasma insulin, enteroglucagon, neurotensin and the release of intestinal CCK (Sagor et al., 1985) may influence intestinal adaptation.

Growth Hormone

Growth hormone is a potent anabolic agent that dramatically affects carbohydrate, protein and lipid metabolism (Lehman and Cerra, 1992; Byrne et al., 1993). Growth hormone is secreted, under the control of neuroendocrine peptides, by the anterior pituitary gland intermittently every 6 - 8 hours (Barber et al., 1989). Stress, exercise, deep sleep, or a decrease in serum glucose are known to increase growth hormone, whereas the aging process and parenteral nutrition have been implicated in its decrease (Barber et al., 1989). Growth hormone affects many biochemical processes in the body including the promotion of reduced carbohydrate uptake in the muscle, deposition of lean muscle mass (Ziegler et al., 1992), increased total body and liver protein synthesis (Lehmann et al., 1990; Clemmons et al., 1987; Snyder et al., 1988) and fat mobilization and oxidation with increased ketogenesis (Ward et al., 1987). In addition, growth hormone increases the transport and reduces the oxidation of amino acids (Lehman and Cerra, 1992). However, adverse effects such as hyperglycemia with or without insulin resistance, fluid retention, headache, weakness and localized muscle pain have been reported during growth hormone administration (Lehman and Cerra, 1992; Ziegler et al., 1992; Lehmann et al., 1990; Ward et al., 1987).

Exogenous administration of growth hormone has been shown to influence intestinal adaptation after extensive intestinal resection in animals (Shulman et al., 1992; Hart et al., 1987). Growth hormone is also known to increase colonic mass and biomechanical strength (Christensen et al., 1990), increase water and sodium absorption (Mainoya, 1982) and appears to regulate amino acid absorption (Inoue et al., 1994). In humans with short bowel syndrome, the combined administration of exogenous growth hormone, glutamine and a diet high in complex carbohydrates and fiber and low in fat results in enhanced nutrient absorption from the remnant bowel (Byrne et al., 1995). Further investigation is merited to assess the efficacy of growth hormone therapy in patients with short bowel syndrome.

Insulin-like growth factors

The insulin-like growth factors (IGFs) 1 and 2 are small homologous peptides related to insulin by structure and function (Herington, 1991). Both IGFs serve as powerful mitogens for a variety of mammalian cell types. IGF-1, previously known as somatomedin C, is thought to mediate the growth-promoting effects of growth hormone, while the role of IGF-2 appears to primarily involve fetal growth (Herington, 1991).

The liver is the principal site of production of IGFs; however, the IGFs can be produced locally and may be important for local or paracrine actions. Both of the IGF-1 and IGF-2 genes are expressed in the adult rat intestine (Lund et al., 1986). *In situ* hybridization of human fetal intestine has localized both IGFs mRNA in the intestinal lamina propria (Lobie et al., 1990). Although the non-hepatic production of IGF-1 is generally assumed to be under local control, the presence of receptors for growth hormone throughout the gastrointestinal tract supports the concept that pituitary regulation of local IGF-1 synthesis also occurs (Lobie et al., 1990).

Endogenous IGF-1 functions at a local and systemic level to increase amino acid uptake, cellular proliferation and differentiation, and suppress protein degradation (Froesch et al., 1985). Parenteral administration of high dose IGF-1 or lower doses of the synthetic analogue des-(1-3) IGF-1 has been shown to promote growth of the gastrointestinal tract (Lemmey et al., 1991). Following MSBR, the level of IGF-1 mRNA in the residual bowel increases, whereas the level of insulin-like binding protein-3 (IGFBP-3) drops precipitously (Albiston et al., 1992; Mantell et al., 1993). As IGFBP-3 modulates the actions of IGF-1 *in vivo*, a decrease in ileal IGFBP-3 synthesis after MSBR may increase the local IGF-1 bioactivity. The administration of IGF-1 following intestinal resection augments morphological adaptation (Lemmey et al., 1991; Mantell et al., 1993; Vanderhoof et al., 1992) and colonic mucosal proliferation and water absorption (Mantell et al., 1995). It would seem likely that IGF-1 does play a role in mediating the adaptation seen after MSBR. This effect is believed to be paracrine in nature, perhaps involving communication between the mesenchymal and epithelial compartments (Taylor and Fuller, 1994).

Epidermal Growth Factor

Epidermal growth factor (EGF), a 53-amino acid polypeptide, has been associated with the regulation of a wide variety of physiologic and pathophysiologic processes including cellular growth (Gospodarowicz, 1981), tissue repair (Chabot et al., 1983), and neoplasia (Bans-Schlegel and Quintero, 1986). EGF is a potent mitogen (Richman et al., 1976), both *in vitro* and *in vivo*, for a variety of cells, including the rapidly renewing small intestinal cells (Marti et al., 1989). A major source of EGF is the submaxillary gland, however synthesis also occurs in the Brunner's glands of the duodenum and the Paneth cells of the small intestine (Skov-Olsen and Nexo, 1983).

EGF is present in moderately large quantities in breast milk, suggesting a role in the growth and differentiation of the gastrointestinal tract. In support of this hypothesis, exogenous administration of EGF appears to be required for development and maintenance of the intestinal epithelium (Al-Nafussi and Wright., 1982; Ulshen et al., 1986; Goodlad et al., 1987c, 1988; Bragg et al.,1990). Infusion of EGF into the ileum of adult rats resulted in more than a 200% increase in the ileum mean mucosal ODC activity, more than a 100% increase in mean deoxyribonucleic acid specific activity and crypt labelling index, and a 25% increase in mean DNA content of the mucosa. The local infusion of EGF into the ileum was shown to effect a distant segment as all measurement except DNA content increased in the jejunum. Furthermore, the stimulation of mucosal ODC activity by intraluminal administration of EGF was dose-dependent, reaching a maximal level within the dose range studied. Thus, the presence of EGF leads to substantial increases in ODC whose presence is obligatory for the post-resection increase in proglucagon-derived peptides (Ulshen et al., 1986). In a later study, Goodlad and associates (1988) confirmed that EGF had a significant effect on intestinal epithelial proliferation throughout the gut of parenterally fed rats, but it could not fully compensate for the lack of luminal contents. Bragg and collegues (1990) have also found that infusion of EGF prevents the TPN-induced intestinal hypoplasia and suggests this may be due to maintenance of mucosal proliferative activity and brush border enzyme activity.

As EGF has been shown to exert trophic effects on the small intestinal mucosa, the changes in mucosal substrate consumption that complement this cellular proliferation were explored. Schwartz and Storozuk (1988) found a significant increase in substrate absorption following systemic and luminal exposure to EGF. In addition, the subcutaneous administration of EGF led to a two-fold upregulation of glutamine transport activity in jejunal brush border membrane vesicles. Kinetic studies of the glutamine transporter showed that the increase in transport was the result of a 70% increase in V_{MAX} which likely indicates an increase in *de novo* biosynthesis of transporter proteins following the EGF administration (Salloum et al., 1993). The application of EGF to the jejunum *in vivo* or *in vitro* upregulates glucose-coupled Na^+ absorption (Schwartz and Storozuk, 1985; Opleta-Madsen et al., 1991) and microvillus brush border surface area within 30 minutes, implying a role in the acute regulation of intestinal transport (Hardin et al., 1993).

EGF has been shown to enhance both morphological and functional indices in the small intestine, however the effect of supplemental EGF on the process of intestinal adaptation is largely unknown. Following proximal intestinal resection in rabbits, long-term treatment with EGF increased maltase activity and dramatically upregulated glucose absorprtion. These functional adaptations were not associated with hyperplasia identifying EGF as a modulator of nutrient transport function specifically (O'Loughlin et al., 1994). However, EGF administration following MSBR in rats resulted in the normal structural adaptive responses (Chalet et al., 1994). Future studies examining the effect of EGF enriched parenteral solutions on the structural and functional adaptations to massive small bowel resection are warranted.

Polyamine synthesis

Polyamine synthesis within the gastrointestinal tract appears to be mandatory for the initiation of the rapid cellular growth and differentiation that occurs during intestinal adaptation to MSBR. The decarboxylation of the precursor, ornithine, by ornithine decarboxylase (ODC) is the first and rate-limiting step in polyamine biosynthesis. In quiescent cells the activity of ODC is low, however it increases markedly during the initial stages of cell proliferation and differentiation (Tabor and Tabor, 1984). This elevation in ODC is often one of the earliest events during the transition from quiescence to proliferation, is associated with the rapid accumulation of cellular polyamines (Pegg and McCann, 1982), and is believed to directly mediate cellular transformation as a potential protooncogene (Auvinen et al., 1992; Wang et al., 1993).

Luk and Baylin (1983) demonstrated that increased mucosal ODC activity and increased polyamine content are intimately associated with the rapid increase in cell proliferation during the adaptive response of the residual intestine after MSBR. Following a 50% intestinal resection, ODC increased 170-fold compared to a 9-fold increase in transected controls. Furthermore, indices of mucosal cell proliferation, including new DNA synthesis, crypt cell proliferation, and crypt cell labelling index, were all highly correlated with the time course of the increase in ODC activity.

To determine if the intestinal mucosal adaptation process is directly dependent upon polyamine biosynthesis, Luk and Baylin (1984) used the specific irreversible ODC inhibitor, α-difluoromethyl ornithine (DFMO) to suppress the increases in intestinal mucosal ODC activity and to assess the resultant effects on intestinal adaptation.

Suppression of ODC activity with DFMO was associated with an 87% suppression of DNA synthesis, and complete abolition of intestinal adaptation, as manifested by the absence of intestinal weight gain, increases in mucosal thickness or increase in crypt cell production *in vivo*. More recently, Taylor and associates (1992) observed a modest 1.3-fold increase in ODC mRNA levels after MSBR, which is present at 18 hours and remains relatively constant thereafter. Their findings were confirmed by Rountree and colleagues (1992) who noted that the changes in ODC mRNA levels following MSBR were independent of luminal nutrition.

Proglucagon-derived Peptides

Proglucagon-derived peptides are a group of previously ill-defined glucagon-like immunoreactive peptides, which were identified primarily in the ileum and colon of various species (Larsson et al., 1975). Proglucagon is a 160 amino acid polypeptide encoded by the glucagon gene (Bell et al., 1983b). Molecular studies have demonstrated that both the rat and the human have single copy glucagon genes (Bell et al., 1983a; Heinrich et al., 1984) originating from the same proglucagon mRNA molecule found in both the intestine and pancreas (Novak et al., 1987). However, within each of these organs proglucagon undergoes differential processing (Figure 1.2), so that the major product in the pancreas is glucagon, whereas those in the gastrointestinal tract include glicentin [corresponding to amino acids 1-69; PG-(1-69)], a C-terminal extended form of glucagon [oxyntomodulin; PG-(33-69)], GLP-1 [PG-(78-108)], GLP-2 [PG-(126-158)], and intervening peptide-2 [PG-(111-123)] (Mojsov et al., 1986; Orskov et al., 1986, 1987). Prohormone convertases, PC1 (also known as PC3) and PC2 are calcium-dependent serine proteases that are responsible for endoproteolytic processing of prohormones found in neuroendocrine cells throughout the body (Steiner et al., 1996). These prohormone convertases cleave at lysine-arginine or arginine-arginine sequences to generate peptides which have the C-terminal end removed by the exopeptidase, carboxypeptidase E (Steiner et al., 1996). Using transformed cell lines, it appears that PC1 is responsible for the processing of proglucagon to glicentin, oxyntomodulin, GLP-1 and GLP-2 in the transfected cells (Rouillé et al., 1995; Dhanvantari et al., 1996; Rothenberg et al., 1996). This organization is consistent for the human (Bell et al., 1983a), rat (Heinrich et al., 1984), bovine (Lopez et al., 1983) and guinea-pig (Seino et al., 1986) sequences. However, angler fish (Lund et al., 1982), catfish (Andrews and Ronner, 1985), and chicken (Hasegawa et al., 1990) proglucagon contain only one GLP.

The L cells in which proglucagon is processed arise from "pluripotent" stem cells which also give rise to enterocytes, goblet cells and Paneth cells (Potten and Loeffler, 1990). The density of the L cells increase from duodenum to colon in a number of mammalian species (Evans and Potten, 1988). L cells are preferentially located in the crypts of Lieberkuhn (Ravazzola et al., 1979) and possess a longer turnover time than the enterocytes (Evans and Potten, 1988). The enteroendocrine L cells are triangular in shape, with their apex in contact with the lumen and their secretory granules predominantly clustered at the basolateral surface. The disposition of secretory granules in the L cells at the basolateral surface is believed to facilitate the release of peptides into the circulation in response to luminal signals (Nilsson et al., 1991; Roberge and Brubaker, 1991). Proglucagon gene expression, biosynthesis, and secretion is regulated by a cAMP-dependent pathway (Drucker and Brubaker, 1989). It is unknown whether nutrient signals increase L cell cAMP; however, the effects appear to be mediated via several second messenger systems (Brubaker, 1988). Using a fetal intestinal cell monolayer culture, Brubaker and Vranic (1987) have demonstrated that several peptides including gastric inhibitory peptide, gastrin releasing peptide, calcitonin gene related peptide can regulate proglucagon-derived peptide secretion. However, PYY, neurotensin, VIP, and CCK are amongst those without an effect (Brubaker, 1991).

There is some understanding of the physiological role of pancreatic-derived glucagon, but that of the intestinal glucagon gene products are only starting to be understood. In models where intestinal adaptation must occur, various researchers have noted enteroglucagon immunoreactivity to be increased (Jacobs et al., 1981; Al-Mukhtar et al., 1983; Bristol and Williamson, 1988). Similarly, in diseases such as celiac disease, where an adaptive response occurs, plasma enteroglucagon levels are elevated (Besterman et al., 1978). Finally, villous hyperplasia was reported in a patient with a renal enteroglucagonoma (Gleeson et al., 1971); intraperitoneal injection of tumour extract resulted in intestinal enlargement in mice (Dowling, 1982). Albeit circumstantial, these observations have provided evidence that enteroglucagon is trophic for the small bowel and may mediate the humoral component of the adaptive response (Bristol and Williamson, 1988).

The insulinogenic relevant form of GLP-1 is not the full 1-36 amide molecule produced by pancreatic α-cells, but rather the truncated 7-37 molecule resulting from a monobasic cleavage in intestinal L cells, which is amidated to a 7-36 amide. GLP-1 is a potent inhibitor of pentagastrin-induced gastric acid secretion via stimulation of cAMP production (Schmidtler et al., 1991). However, much recent attention has been focused on its insulinotrophic (incretin) effect (for review, see Drucker, 1990; Goke et al., 1991). In addition to its role as an incretin in several species including man, infusion of supraphysiological levels of GLP-1 (7-36 amide) into subjects with non-insulin dependent diabetes mellitus (NIDDM) improved their response to exogenous insulin. Unlike gastric inhibitory peptide, GLP-1 (7-36 amide) maintains its insulintropic activity in NIDDM (Nauck et al., 1993). The incretin effect displayed by GLP-1 (7-36 amide) appears to be mediated via inhibition of the sulphonylurea-sensitive potassium channel in the pancreatic β-cell, rendering the cells glucose competent (Holz et al., 1993).

Despite its sequence conversion within many animal species, a role for GLP-2 as an inducer of basolateral glucose transport has only recently been described (Cheeseman and Tsang, 1996). Glicentin and oxyntomodulin are believed to play a trophic role in the intestine. Plasma levels of both glicentin and oxyntomodulin are increased following a 50% proximal, but not distal, intestinal resection (de Segura et al., 1994). It is of interest that a greater capacity for intestinal adaptation exists when the ileum is conserved (Appleton et al., 1987). This phenomena might be related to the preservation of L cells in such models.

The past decade has brought the molecular characterization of proglucagon which enables a closer examination of the changes occurring during intestinal adaptation. Taylor and associates (1990) were the first to analyze the changes in gene expression during the adaptive response. Following MSBR, proglucagon mRNA in the residual terminal ileum are increased three-fold, peaking two days following surgery. This increase in proglucagon gene expression is partly dependent on luminal nutrition, as demonstrated by significantly higher levels in fed rats as compared to fasted rats at 48 hours post-MSBR (Taylor et al., 1990). Upregulation of proglucagon gene expression following MSBR has been reported by other researchers (Rountree et al., 1992; Bilchik et al., 1995). Although much less abundant, parallel changes were also observed in the mRNA levels of CCK and PYY (Taylor et al., 1992; Fuller et al., 1993), but not in the levels of several other genes indicating specificity of the observed responses. The rise in proglucagon mRNA after proximal intestinal resection is not inhibited by DFMO, despite inhibition of intestinal adaptation. This study indicates that proglucagon-derived peptides are possible modulators of adaptive bowel growth but cannot stimulate growth when ODC activity is inhibited (Taylor et al., 1992).

In situ hybridization and electron microscopy has revealed that increased proglucagon gene expression represents an increase in the synthetic activity of the L cell rather than an increase in L cell number (Fuller et al., 1993; Buchan et al., 1985). In addition, following MSBR the normal relatively basal disposition of the L cells in the crypt-villus unit is lost, as enterocyte proliferation leads to L cell displacement onto the villi (Fuller et al., 1993).

Monoclonal antibodies to 'enteroglucagon' have been used to show minimal effects on the adaptive response following MSBR (Gregor et al., 1987; Gregor et al., 1990). However, this data is unconvincing as the ability of the antisera to neutralize the relevant proglucagon peptide product remains unknown. Future studies will require more precise definition of the proglucagon-derived peptide being examined in an effort to identify the proglucagon-derived peptide responsible for mediating the adaptive response (Rountree et al., 1992).

Secretion of proglucagon-derived peptides in response to nutrient ingestion has been demonstrated. Plasma GLP-1 concentration rises within 15 - 30 minutes in response to oral ingestion of a mixed meal and remains elevated for several hours (Kreymann et al., 1987; Gutniak et al., 1992; Elliott et al., 1993). Luminal glucose perfusion of the ileum or colon stimulates GLP-1 release (Orskov et al., 1986; Shima et al., 1990; Plaisancié et al., 1995). Ingestion of fermentable fibers have been recently noted to increase ileal proglucagon gene expression, plasma GLP-1 (Reimer and McBurney, 1996), glicentin and oxyntomodulin concentration (Gee et al., 1996), presumable due to the fermentative end products - SCFAs. Using a colonic perfusion model, no effect of SCFAs on GLP-1 secretion were noted (Plaisancé et al., 1995). This may be due to site specific regulation differences in the ileum and colon or experimental aberration as other inconsistencies exists. The intracolonic perfusion of oleic acid did not modify GLP-1 release (Plaisancé et al., 1995) whereas oleic acid in isolated perfussed rabbit left colon induced glicentin and oxyntomodulin release (Ballantyne et al., 1989). Similarly, luminal stimulation by a fat emulsion in the rat ileum evoked a delayed increase in plasma glicentin and oxyntomodulin levels *in vivo* (Roberge and Brubaker, 1991) and in fetal rat intestinal cultures (Rocca and Brubaker, 1995). The release of proglucagon-derived peptides is believed to occur synchronously (Orskov et al., 1986).

Recently, Bamba and associates (1994) reported that plasma glicentin and oxyntomodulin levels were further increased following proximal small bowel resection in rats consuming a pectin-containing elemental diet than in rats consuming a fiber-free elemental diet. The effect of systemically administered nutrient on proglucagon expression following MSBR is unknown.

Peptide YY

Peptide YY (PYY), a 36 amino acid peptide, is co-localized with the proglucagon-derived peptides in the L cells of the colon and ileum (Ali-Rachedi et al., 1984; Bottcher et al., 1984). Plasma PYY levels parallel the rise in proglucagon mRNA levels following MSBR and have therefore been suggested to be a possible humoral growth factor (Adrian et al., 1987; Goodlad et al., 1989). However, more recent studies have suggested that its role in intestinal adaptation is that of an 'ileal brake' (Spiller et al., 1988; Goodlad et al., 1990) to decrease motility and allow increased contact time between nutrients and the epithelium (Goodlad et al., 1990). PYY is released from the L cells in a dose-dependent fashion following colonic perfusion with the SCFAs, acetate and n-butyrate (Longo et al., 1991) suggesting that L-cells secrete peptides in response to SCFAs.

CELLULAR REGULATORS OF INTESTINAL PROLIFERATION

Cellular proliferation is achieved over the course of a series of defined events - initial signaling, followed by the induction of a program of gene expression which ultimately culminates in DNA synthesis and cell division (Pardee, 1989). The initial signaling phase is rapid, beginning with a 2-3 hour ‘commitment’ period, after which proliferation proceeds in a relatively autonomous manner (Crabtree, 1989). During this commitment period the expression of a number of proto-oncogenes, or ‘early response genes’, is activated (Ullman et al., 1990). This altered gene expression is essential for the initiation of proliferation and may therefore be considered a crucial regulatory point, both during entry into, and subsequent progress through, the cell cycle (Pardee, 1989; Crabtree, 1989; Ullman et al., 1990).

There has been particular interest in the class of early response genes that encode DNA binding proteins which regulate gene transcription within the nucleus, as these may represent part of the final common pathway of cell cycle control (Kerr et al., 1988). The expression of certain early response genes, including c-*myc*, c-*jun* and c-*fos* increase rapidly but transiently in response to cellular stimulation by mitogens or growth factors (Kelly et al., 1988; Muller et al., 1984). The expression of early response genes may trigger secondary changes in gene activity and is thought to ultimately activate complex genetic programs, such as those controlling cell growth, differentiation or response to environmental information (Figure 1.3).

c-myc

The earliest discovered nuclear oncogene, c-*myc*, encodes for a nuclear phosphoprotein that functions as a transcriptional factor controlling cell division, differentiation and apoptosis (Marcu et al., 1992, Varmus, 1984). The Myc protein interacts with Max, a basic helix-loop-helix zipper protein, and the Myc-Max heterodimeric complexes stimulate transcription and cell proliferation (Gu et al., 1994). Decreased expression of c-*myc* gene by the antisense oligomer complementary to the c-*myc* initiation codon and downstream sequences prevents the transition from the G1 phase to the S phase in the cell cycle and is associated with inhibition of cell proliferation in a variety of cell types (Heikkila et al., 1987; Holt et al., 1988).

One of the physiological functions of the c-*myc* genes is the intracellular transduction and multiplication of growth-promoting signals derived from stimulated growth-factor receptors at the cell surface (Rottleb et al., 1995). In resting cells or in terminally differentiated cells, c-*myc* expression is shut off. Similarly, *in vitro* systems are commonly used in differentiation assays and differentiation is typically accompanied by an early reduction of c-*myc* expression (Westin et al., 1982; Lachman et al., 1985). The decrease in c-*myc* expression is essential, allowing the subsequent steps of differentiation to take place. Differentiation can be blocked by constitutive expression of c-*myc* (Marcu et al., 1992) or induced by suppression of c-*myc* translation through anti-sense RNA or anti-sense oligonucleotides. Following intestinal resection in rats, there is a rapid and marked induction of c-*myc* expression which culminates in stimulation of DNA synthesis in ileal crypt epithelial cells 24 hours later (Sacks et al., 1995). This rapid c-*myc* response was in the absence of enteral nutrition, thus future studies should focus on the modulation of early response genes by specific nutrients in the first few hours following intestinal resection.

c-jun and c-fos: The AP-1 Transcription Factor

c-*jun* and c-*fos* encode for DNA binding proteins that dimerize to form the AP-1 (Activator Protein-1) transcription factor (de Groot et al., 1990). AP-1 DNA binding regulates multiple genes involved in cellular growth and differentiation (de Groot et al., 1990). The *Fos*- and *Jun*-related proteins form homodimeric and/or herodimeric complexes that are stabilized by noncovalent interactions between the leucine-zipper domains present in all *Fos* and *Jun* proteins (Bohmann et al., 1989; Angel et al., 1988; Curran and Franza, 1988; Rauscher et al., 1988; Bohmann and Tjian, 1989). *Jun-Jun* or *Jun-Fos* dimerization has been shown to be necessary for DNA binding and transcriptional activation (Halazonetis et al., 1988; Nakadeppu et al., 1988; Turner and Tjian, 1989). AP-1 binding sites are frequently found in promoters or enhancers of genes that are inducible by a variety of extracellular signals or growth factors indicating that the transcriptional activation of genes in response to trophic stimuli is indeed exerted by AP-1. Such mechanisms could implicate AP-1 in the induction of the 'first wave' of gene transcription that follows cell stimulation.

Nutrient Regulation of c-myc, c-jun and c-fos

Studies of fasted and fed animals have demonstrated that intraluminal nutrients stimulate the expression of early response genes. c-*fos* and c-*jun* expression is upregulated in the proliferative period that is induced by intraluminal nutrients following fasting (Hodin et al., 1994; Holt and DuBois, 1991). Using a nutrient deprivation model, IPEC-J2 cells treated with glutamine for 20 minutes to 12 hours showed c-*jun* induction at 20 minutes followed by a rapid decline to baseline (Kandil et al., 1995). This data suggests that the initial step in glutamine-stimulated proliferation may involve c-*jun* expression.

In vitro work has demonstrated that the SCFA, butyrate alters c-*myc* (Rabizadeh et al., 1993; Rottleb et al., 1995), c-*fos* (Souleimani and Asselin, 1993) and c-*jun* (Nishina et al., 1993, Rabizadeh et al., 1993) expression thereby modulating proliferation and differentiation. Until recently, butyrate was believed to lack biological effects on oncogene expression *in vivo* due to it's rapid metabolism (Miller et al., 1987). However, earlier this year Velázquez and associates (1996b) reported that the injection of butyrate into the colonic lumen increases c-*Jun* protein abundance in association with changes in crypt compartment-specific proliferation in normal rat colon *in vivo*. The effect of systemic SCFA administration on early response genes following the trophic stimuli of intestinal resection is unknown.

SUMMARY

As revealed by the discussion of the preceding studies, a complex relationship exists among many of the factors involved in intestinal adaptation. Descriptive commentaries of the actual process of intestinal adaptation are somewhat unclear as the structural and functional changes appear to occur independently. Additional research regarding the impact and interaction of gut specific fuels and humoral factors is warranted in an attempt to achieve mechanistic understanding. Ultimately, the knowledge derived will provide practical information enabling the revision and improvement of current parenteral solutions for patients with intestinal disease or injury.

AIMS AND SCOPE OF RESEARCH

Following massive small bowel resection, the remnant intestine undergoes morphological (i.e. dilation, lengthening and thickening) and functional (i.e. hyperproliferative, digestive and absorptive) adaptive changes (Wilmore et al., 1971; Sigalet et al., 1990). Prior to these adaptive events, patients with short bowel syndrome present with diarrhea, weight loss, electrolyte abnormalities and multiple nutritional deficiencies (Dudrick et al., 1991). Various other metabolic consequences of surgery occur. Accelerated skeletal muscle proteolysis and translocation of amino acids from skeletal muscle to visceral organs follows surgical stress (Souba and Wilmore, 1983). In addition, surgery itself has been shown to have immunosuppressive effects by decreasing helper and suppressor lymphocyte populations (Hansbrough et al., 1984), granulocyte function (Christon, 1985), NK cell cytotoxicity (Pollack et al., 1991) and delayed hypersensitivity skin-test response (Johnson et al., 1979).

The successful role of TPN in providing nutritional support for these patients somewhat obscures the reduction in intestinal adaptation and mucosal atrophy that occurs if the intestine is robbed of the stimulus of continued enteral nutrition (Booth, 1994; Johnson et al., 1975a; Wilmore et al., 1988). TPN is also associated with immunosuppression as reflected by an increased incidence of infections in TPN- versus enterally-fed patients (Kudsk et al., 1992; Mainous et al., 1991; McGeer et al., 1990; Moore et al., 1992). The mechanism for increased susceptibility to infection and immunosuppression associated with TPN is not known, however detrimental changes have been reported in both nonspecific (Cleary and Pickering, 1983; Fraser et al., 1983; Hawley and Gordon, 1976;

Monson et al., 1988; Pollack et al., 1991) and cell-mediated immunity (Gogos et al., 1990; Kinsella et al., 1990; Sedman et al., 1991; Shou et al., 1994; Wolfe et al., 1982). The inability of the immune system to resist infection can lead to hypermetabolism and sepsis. These multiorgan abnormalities have prompted the addition of specific nutrients to current parenteral nutrient formulations in an attempt to enhance outcomes in the critically ill.

Current parenteral formulations do not provide nutrients specific to the GI tract. The addition of SCFAs to TPN has been shown to prevent TPN-associated mucosal atrophy (Koruda et al., 1990) and enhanced morphological markers of adaptation to small bowel resection in rats (Koruda et al., 1988b). The effect of SCFA-supplemented TPN on other organs known to be affected by intestinal resection, such as the skeletal muscle and immune systems, is unknown. Additional research regarding the impact and interaction of gut specific fuels and humoral factors is warranted in an attempt to achieve mechanistic understanding. Ultimately, the knowledge derived will provide practical information enabling the revision and improvement of current parenteral solutions for patients with intestinal disease or injury.

The objectives of this thesis research are:

1) to identify mechanism(s) by which SCFAs are trophic to the gastrointestinal tract;
2) to provide insight into regulatory mechanisms of intestinal adaptation, in both resected and normal nonresected intestine, and;
3) to determine if SCFA supplementation of TPN influences the multiorgan response to surgical trauma.

These aims will be achieved by testing the following hypotheses:

Hypothesis 1: SCFA supplemented-TPN enhances structural and functional aspects of intestinal adaptation following intestinal resection, and is associated with immunoenhancement and reduced skeletal muscle catabolism.

Chapter 2 established the TPN / intestinal resection model in rats and corroborates the effect of SCFA-supplemented TPN on structural aspects of intestinal adaptation. Chapter 3 describes SCFA induced changes in functional markers of intestinal adaptation at the cellular level. Chapter 4 investigates the effect of systemic SCFAs on immune function following surgical trauma. Chapter 5 examines SCFA-supplemented TPN on skeletal muscle wasting following intestinal resection. Chapter 6 provides preliminary evidence regarding the mechanism by which SCFAs mediate the multiorgan response to SCFAs following intestinal resection.

Hypothesis 2: Systemic SCFAs rapidly modulate the gene expression of factors believed to be essential intestinal regulation.

Chapter 2 investigates the effect of SCFA-supplemented TPN on the expression of enterotrophic genes (i.e. proglucagon, ornithine decarboxylase) following intestinal resection. Chapter 7 describes the enteroplastic response and associated gene expression to SCFAs in parenterally-supported rats with an intact small intestine. Chapter 8 examines acute enteroplastic responses and expression of early response genes due to systemic SCFA administration in normal rats.

Chapter 9 summarizes the testing of these hypotheses and discusses the knowledge derived while accomplishing the specific objectives.

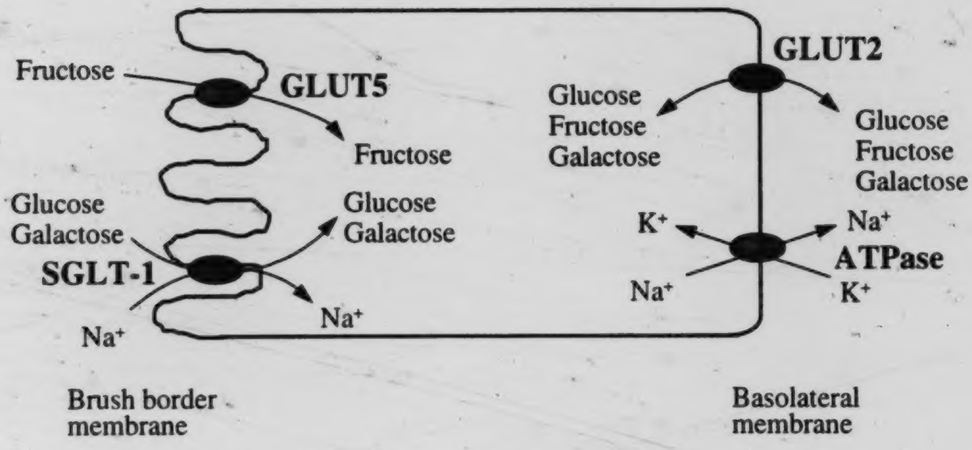
Fructose
GLUT5
Fructose
Glucose
Galactose
SGLT-1
Na+
Glucose
Galactose
Na+
GLUT2
Glucose
Fructose
Galactose
Glucose
Fructose
Galactose
K+
Na+
Na+
ATPase
K+
Brush border membrane
Basolateral membrane

Figure 1.1 Enterocyte Hexose Transport.

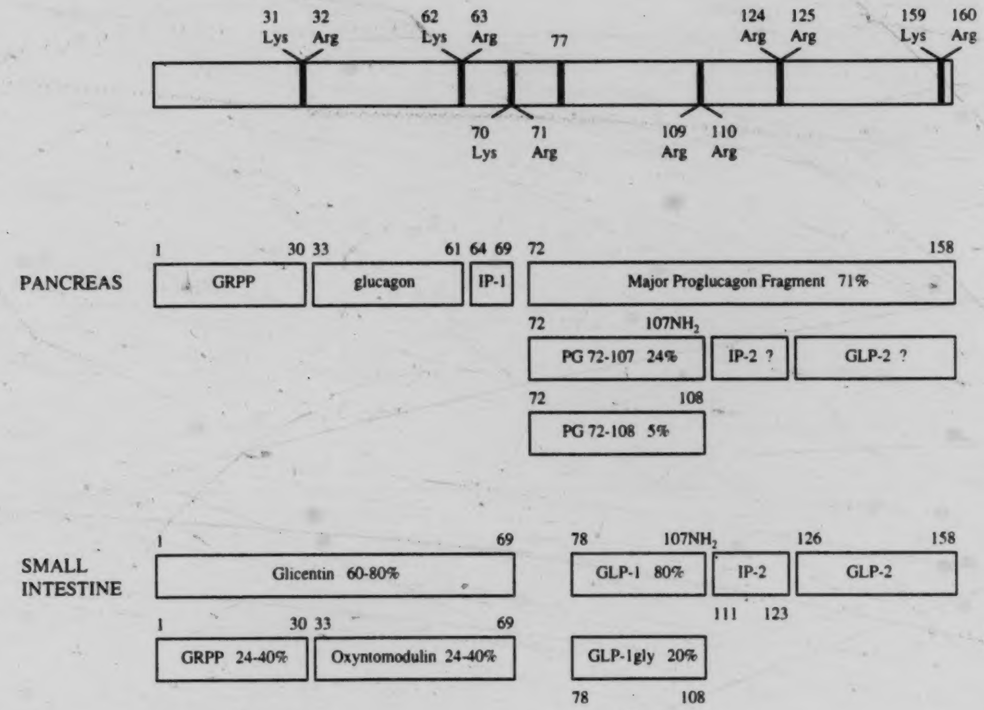
31 Lys
32 Arg
62 Lys
63 Arg
77
124 Arg
125 Arg
159 Lys
160 Arg
70 Lys
71 Arg
109 Arg
110 Arg
PANCREAS
1
30
33
61
64
69
72
158
GRPP
glucagon
IP-1
Major Proglucagon Fragment 71%
72
107NH$_2$
PG 72-107 24%
IP-2 ?
GLP-2 ?
72
108
PG 72-108 5%
SMALL INTESTINE
1
69
78
107NH$_2$
126
158
Glicentin 60-80%
GLP-1 80%
IP-2
GLP-2
111
123
1
30
33
69
GRPP 24-40%
Oxyntomodulin 24-40%
GLP-1gly 20%
78
108

Figure 1.2 Schematic Representation of the Post-Translational Processing of Proglucagon as it Occurs in the Pancreas and the Intestinal L-cells.
The numbers indicate positions of amino acid residues in proglucagon. IP, intervening peptide; GRPP, glicentin-related pancreatic polypeptide.

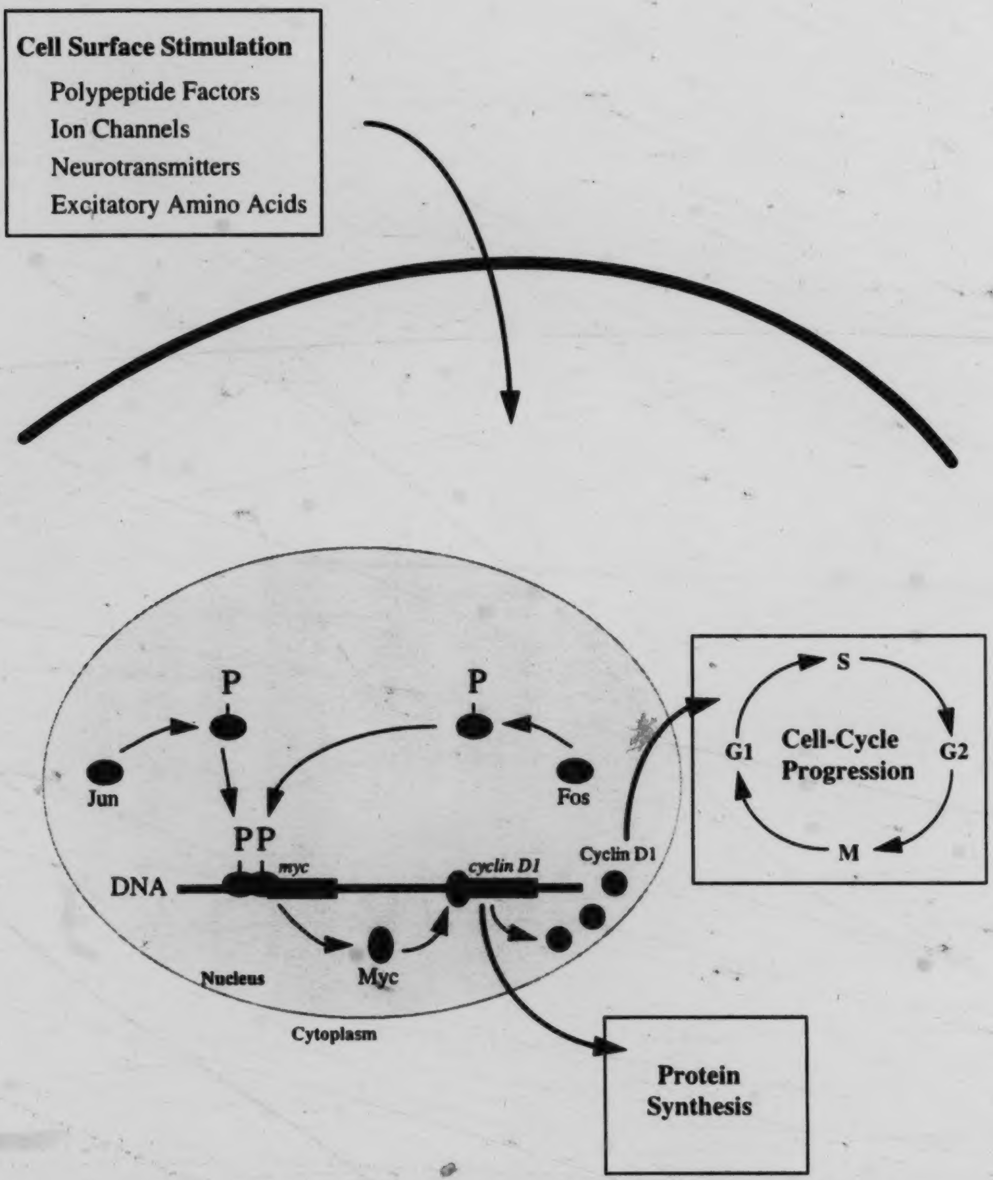
Cell Surface Stimulation
Polypeptide Factors
Ion Channels
Neurotransmitters
Excitatory Amino Acids
P
P
Jun
Fos
PP
myc
cyclin D1
Cyclin D1
DNA
Myc
Nucleus
Cytoplasm
S
G1
Cell-Cycle Progression
G2
M
Protein Synthesis

Figure 1.3 Cellular Proliferation through a Series of Proteins Encoded by Early Response Genes

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CHAPTER 2

SHORT-CHAIN FATTY ACIDS INCREASE PROGLUCAGON AND ORNITHINE DECARBOXYLASE MESSENGER RNAS FOLLOWING INTESTINAL RESECTION IN RATS[1,2,3]

INTRODUCTION

Massive small bowel resection results in "short bowel syndrome" which is clinically characterized by diarrhea, weight loss, electrolyte abnormalities and multiple nutritional deficiencies (Dudrick et al., 1991). Advances with home total parenteral nutrition (TPN) programs have proven to be life sustaining for many patients (Purdum and Kirby, 1991; Tilson, 1980; Daniano et al., 1989; King et al., 1987), however the systemic administration of nutrients inhibits both morphological (Johnson et al., 1975; Levine et al., 1974; Hosada et al., 1989; Rossi et al., 1993; Inoue et al., 1993; Miura et al., 1992) and functional (Levine et al., 1974; Inoue et al., 1993; Miura et al., 1992) aspects of intestinal adaptation. Ultimately, it is the adaptive ability of the remnant bowel to compensate for the absence of the resected portion that will determine the patient's prognosis.

The mechanism by which exogenous nutrients enhance intestinal adaptation is unclear but are thought to act either directly by providing protein and energy for the mucosal cells of

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[1] A version of this chapter has been published. Tappenden KA, Thomson ABR, Wild GE, McBurney MI. JPEN 1996;20:357-362.

[2] Awarded, in part, the American Institute of Nutrition/Procter & Gamble Graduate Student Research Award at the Federation of American Societies for Experimental Biology, April 1995, Atlanta, Georgia and published, in part, in abstract form, The FASEB Journal 1995;9:4999.

[3] Awarded, in part, the Mary Ecclestone Graduate Student Award of the Canadian Society of Nutritional Sciences at the 38th Annual Meeting of the Canadian Federation of Biological Societies, June 1995, Saskatoon, Saskatchewan, and published, in part, in abstract form Can Fed Proc 1995;38:322J.

the small intestine (Philpott et al., 1993) or indirectly by triggering the release of secondary factors (Hughes et al., 1978; Weser et al., 1977; Gelinas and Morin, 1980; Goodlad et al., 1987; Bristol and Williamson, 1988; Dworkin et al., 1976). Enteroglucagon is a group of previously ill-defined glucagon-like immunoreactive peptides, which are secreted from enteroendocrine L-cells found predominantly in the ileum and colon of various species (Larsson et al., 1975). Many studies have established a strong correlation between cell proliferation during intestinal adaptation and elevated plasma levels of enteroglucagon (Sagor et al., 1982; Dowling, 1982; Bloom and Polak, 1982; Sagor et al., 1983; Gornacz et al., 1984; Al-Mukhtar et al., 1982). To further support the hypothesis that proglucagon-derived peptides are enterotrophic hormones, adaptive hyperplasia is associated with increases in intestinal proglucagon mRNA (Hynes et al., 1988; Rountree et al., 1992; Taylor et al., 1990). Rountree and associates (1992) have shown that the sustained increase in proglucagon mRNA following intestinal resection requires luminal nutrition but there is an absence of information regarding the effect of specific nutrients on proglucagon gene expression.

Ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine synthesis, plays an important role in the regulation of intestinal growth (Luk et al., 1980; Luk and Baylin, 1983; Yang et al., 1984; Maudsley et al., 1976; Tabata and Johnson, 1986; Seidel et al., 1984; Wang et al., 1991; Wang and Johnson, 1991). Following proximal bowel resection, intestinal ODC activity increases very rapidly and is considered to be a marker of the onset of adaptive growth (Luk and Baylin, 1983). In further support of the role of ODC during intestinal adaptation, Luk and Baylin (1984) showed that inhibition of mucosal ODC activity with α-difluoromethyl ornithine, a specific, irreversible ODC inhibitor, completely abolishes markers of intestinal adaptation. It is possible that ODC and subsequent polyamine synthesis may represent a final pathway through which trophic substances, such as enteroglucagon, stimulate intestinal adaptation.

TPN supplemented with short chain fatty acids (SCFAs), the byproduct of dietary fiber fermentation in the colon, has been shown to prevent TPN-associated mucosal atrophy and thus facilitate adaptation to small bowel resection in rats (Koruda et al., 1988; Koruda et al., 1990). It has been postulated that SCFAs may influence small intestinal mucosal proliferation by increasing pancreatic secretions (Harada and Kato, 1983; Al-Mukhtar et al., 1983), small intestinal blood flow (Dowling, 1982) and, although not extensively studied, through stimulation of enterohormone secretions (Koruda et al., 1990). In this study, we sought to determine if systemic SCFA administration enhances intestinal adaptation through regulation of the mRNA abundance of trophic factors, specifically proglucagon and ornithine decarboxylase.

MATERIALS AND METHODS

Animals

Forty-six adult male Sprague-Dawley rats (weighing 236 ± 7 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet and drinking water. The elemental diet was given to minimize the effect of residual dietary fiber fermentation and therefore decrease SCFA production in the gastrointestinal tract prior to the onset of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care.

Surgical Model

Prior to surgery, animals underwent a 12 hour food restriction, were weighed and anesthetized with halothane. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (1981). Subsequently, all animals were subjected to an 80% jejunoileal resection, leaving 1-cm of jejunum distal to the ligament of Treitz and 15-cm of ileum proximal to the ileocecal junction. The 15-cm intestinal segment proximal to the ileocecal valve was measured using a 15-cm length of 3-0 silk suture placed along the anti-mesenteric border of the gently stretched small intestine. Thus, all animals were left with an equivalent length of distal ileum and 1-cm of proximal jejunum. Bowel continuity was restored by an end-to-end jejunoileal anastomosis with interrupted 6-0 silk sutures. The abdomen was closed with interrupted 3-0 dexon sutures. Sterile instruments and aseptic technique were used at all times.

Postoperative Care and Nutrient Solutions

Postoperatively, animals were randomly assigned to receive either standard TPN or TPN supplemented with SCFAs. Within these two diet groups, animals were further randomized to receive the parenteral solutions for either 3 or 7 days. The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain optimal sterile conditions and were filter sterilized before infusion (0.22µm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemicals, St. Louis, MO) in the molar proportions found physiologically in the colon of man (Cummings, 1984) and at concentrations demonstrated by Koruda and associates (1988) to reduce TPN mucosal atrophy following massive small bowel resection. The diets were infused daily to provide 205 nonprotein kJ and 425 mg nitrogen with a nonprotein energy in a volume of 52.8 mLs. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue Preparation

On the third (TPN-3, n=12; SCFA-3, n=11) and seventh (TPN-7, n=11; SCFA-7, n=12) postoperative days, animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of its mesenteric fat, rinsed in ice-cold saline and weighed. A 5-cm section of tissue, located 2-cm distal to the anastomosis, was removed, snap frozen in liquid nitrogen and stored at -72°C for subsequent RNA isolation and quantification. A 2-cm segment of distal ileum

(2-cm proximal to the ileocecal valve) was opened longitudinally, rinsed further with ice-cold saline, blotted dry and the mucosa was completely removed by scraping with a glass slide. The mucosal and submucosal portions were then weighed. An additional 2-cm segment (located 4-cm proximal to the ileocecal valve) was stored at -72°C until assayed for DNA (Prasad et al., 1972) and protein (Lowry et al., 1951).

Northern Analysis

Total RNA was isolated and quantified according to the method of Chomzynski and Sacchi (1987). Northern blot analysis of proglucagon expression was performed as described by Taylor et al. (1990), with minor modifications. Briefly, 15 µg of total RNA was fractionated on a 1.5% (w/v) agarose gel containing formaldehyde (0.66 *M*) and transferred to a positively charged nylon membrane (Boehringer Mannheim, Laval, PQ). The RNA was crosslinked to the membranes by exposure to ultraviolet light.

The 440 basepair proglucagon cDNA probe (Taylor et al., 1990), a gift from Peter J. Fuller of Prince Henry's Institute of Medical Research in Melbourne, Australia, was labelled by nick translation (Random Primers DNA Labelling System, Life Technologies, Burlington, ON) with [^{32}P]-dATP (3000Ci/mmol, Amersham Canada, Oakville, ON). Membranes were prehybridized for 2 hours at 65°C in prehybridization buffer [6 x SSPE (0.18 M NaCl, 0.01 M sodium phosphate, 1 mM EDTA, pH 7.4), 0.1% SDS (wt/vol), 5 x Denhart's solution (0.5 g Ficoll 400, 0.5 g BSA (frakcia v)]. Following prehybridization, membranes were incubated for 16 hours at 68°C in an identical volume of fresh hybridization buffer, but with the addition of the proglucagon [^{32}P]-ATP-labelled cDNA probe.

The 2158 basepair ODC cDNA was labelled with digoxigenin using random primed labelling technique (Feinberg and Vogelstein, 1983). The digoxigenin-labelled ODC cDNA probe detected two major transcripts of 2.6 kb and 2.2 kb (Blackshear et al., 1989). Prehybridization and hybridization techniques were carried out according to optimized standard protocols for use with digoxigenin-labelled cDNA probes (Boehringer Mannheim Canada, 1994).

Following hybridization, the blots were washed once at room temperature in 2 x SSC, 0.1% SDS, and then twice for 20 minutes at 68°C. The blots were exposed to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (Dupont Canada, Mississauga, ON) at -70°C. Relative mRNA concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, Biorad Laboratories (Canada) Ltd., Mississauga, ON). RNA integrity and loading accuracy were confirmed by quantification of the 28S ribosomal unit from the ethidium bromide stained membranes. This technique of using 28S ribosomal units has been used in models of intestinal adaptation to compensate for any loading discrepancies, variations in RNA integrity, or nonspecific changes in RNA abundance (Winesett et al., 1995).

Statistics

The effect of time on body weight was determined for each group by repeated measures analysis of variance (ANOVA). Differences between treatments were determined using a randomized block ANOVA. Sources of variation were blocks (experimental day = 12), diet (d=2), time (t=2) and diet interacted with time. Comparisions within the diet by time interaction were made using student's t-test. Statistical significance was defined as $p \leq 0.05$. Computations were done using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC). All data are presented as mean ± SEM.

RESULTS

Body Weight

All animals received continuous nutrient infusion throughout the study period and grew an average of 2.4 g/day (Figure 2.1). Change in body weight did not differ between diet groups (Day 3, +10 ± 5 g; Day 7, +17 ± 6 g). All animals grew at a rate comparable to nonsurgical, *ad libitum* chow fed controls (data not shown) indicating the adequacy of the nutritional support provided.

Ileal Parameters

The weight of the whole ileum and ileal mucosa were measured to quantitate the extent of adaptive growth which had occurred by 3 and 7 days after surgery. Total ileal weight (mg/cm) was significantly elevated ($p<0.05$) in the SCFA group when compared to the TPN group at both time points (Table 2.2). This change was consistent in both the mucosal and submucosal portions (Table 2.2). Ileal protein concentration was significantly lower ($p=0.0001$) in the TPN group by day 7, but did not differ between diet groups 3 days following surgery (Table 2.3). Ileal DNA ($p<0.03$) and RNA ($p=0.0004$) concentrations were significantly higher in the SCFA groups when compared to the TPN groups at both time points (Table 2.3).

Proglucagon mRNA

Northern analysis confirmed the presence of the 1.2 kb proglucagon mRNA species in the ileal total cellular RNA. As shown in Figure 2.2, ileal proglucagon mRNA abundance was significantly higher ($p<0.05$) in the SCFA group as early as 3 days following surgery. These levels observed at day 3 were sustained until day 7 (Table 2.4).

Ornithine decarboxylase mRNA

Northern analysis confirmed the presence of the 2.6 and 2.2 kb ODC transcripts (Figure 2.3). The level of abundance of ornithine decarboxylase mRNA was greater ($p<0.05$) in the ileum in those animals receiving SCFA supplemented TPN at both time points following surgery (Table 2.4).

DISCUSSION

The morphological and functional responses of intestinal adaptation to jejunoileal resection are well characterized. However little is known about the mechanism regulating this phenomena. Traditionally, two broad categories of regulatory processes have been examined - luminal factors and systemic factors (Philpott et al., 1993). There are substantial bodies of literature supporting the role of each of these regulatory processes however evidence is lacking regarding the interaction of the two, or more specifically the ability of specific nutrients to alter systemic factors.

TPN alimentation provides an ideal model with which to study the role of specific nutrients in intestinal adaptation following resection. The use of TPN following resection allows examination of distinct nutrients while enabling the provision of adequate nutrition support. Massive small bowel resection in rats reliably produces a syndrome characterized by massive prolonged diarrhea, malabsorption of enteral nutrients, negative nitrogen balance and loss of body weight (Roth et al., 1995). Consequently, the use of oral diets in these animals results in malabsorption and malnutrition thus confounding nutrient effects with those of under-nutrition and lower body weight. In addition, the systemic provision of nutrients eliminates direct contact of intraluminal nutrients with the epithelial cells and subsequent secretion of trophic upper gastrointestinal secretions. Finally, the ability of systemically administered nutrients to prevent TPN associated mucosal atrophy thus enhancing intestinal adaptation is of significant clinical relevance.

Total and mucosal weight and ileal DNA, RNA and protein concentrations were used as indices of intestinal adaptation. One week following surgery all adaptive markers were significantly enhanced in the SCFA group. With the exception of ileal protein concentration these adaptive markers were increased at day 3 postsurgery. The increase in DNA as early as day 3 is to be expected as an increase in the incorporation of ^{3}H-thymidine into DNA has been shown as early as 24-36 hours after resection in the rat (Miazza et al., 1982). The delayed increase in ileal protein concentration may support the hypothesis that during adaptation individual enterocytes spend less time in the differentiation zone of the crypts (Menge et al., 1982) and may be functionally immature (Menge and Robinson et al., 1978; Morita et al., 1982). Furthermore, as DNA is a more specific indicator of cellularity than protein, this nonparallel response may indicate that SCFAs cause trophism by initially increasing cell number rather than size.

There is a strong correlation between plasma levels of enteroglucagon and the adaptive growth of the ileum following resection (Dowling, 1982; Bloom and Polak, 1982; Bloom et al., 1979), supporting the possibility that enteroglucagon is an enterotrophic hormone. Rountree and coworkers (1992) showed that sustained increases in intestinal proglucagon mRNA following resection in rats requires luminal nutrition. In their study, the effects of luminal nutrition were compared to those of fasting. It is unclear whether their results suggest a need for luminally provided substrate or the generalized requirement for nutrients during this hypermetabolic postsurgical period. Gee and associates (1996) recently demonstrated that the ingestion of fermentable, nonabsorbed carbohydrate were an important stimulus for the release of enteroglucagon. Reimer and McBurney (1996) reported that the ingestion of dietary fiber significantly increases proglucagon gene expression in rats which are associated with significantly greater plasma concentrations of glucagon-like peptide-1 thirty minutes after an oral glucose gavage. The present study is the first to show that SCFAs upregulate proglucagon mRNA following resection. Physiologically, SCFAs are a logical mediator of enteroglucagon release as the enteroendocrine L cells, which secrete enteroglucagon, are located in the terminal ileum and colon (Bloom, 1980) - a strategic position for monitoring the presence of malabsorbed food and SCFA release. The specific mechanism by which SCFAs increase intestinal proglucagon gene expression is not known but may involve cAMP-dependent pathways (Rocca and Brubaker, 1995).

We found that the adaptive growth at day 3 was associated with increased ileal ODC mRNA suggesting that SCFAs may influence ileal DNA, RNA and protein concentration by increasing ODC mRNA abundance. This may be a reflection of increased gene transcription or mRNA stability. Although not statistically significant, the relative abundance of ODC mRNA in the TPN group over time resembled the rapid increase and subsequent return to normal levels by day 4 reported by Rountree and colleagues (1992). In this study, the sample collection occurred at day 3 which may have been too late to detect this difference as ODC mRNA was reported to have peaked 48 hours after surgery.

The physiological significance of SCFAs ability to maintain the level of ODC until day 7 is unclear but the polyamines synthesized by ODC are thought to stabilize nucleic acids and influence protein synthesis (Das and Kanungo, 1982; Fozard and Koch-Weser, 1982).

This study suggests that intravenous administration of SCFAs upregulate proglucagon and ornithine decarboxylase mRNAs to enhance intestinal adaptation following massive small bowel resection. The proliferative response in this study cannot be attributed to one particular SCFA. However, available evidence suggests that butyrate may be important in inducing these effects. Butyrate is a preferred oxidative fuel (Marsman and McBurney, 1996) and of the three major SCFAs, the effects of colonic mucosal proliferation are thought to be mostly due to butyrate (Sakata, 1987; Kripke et al., 1989). Lupton and Kurtz (1993) reported that amongst the SCFAs examined, the strongest positive correlation with *in vivo* cell proliferation was found with colonic butyrate concentrations. More recently, butyrate has been reported to increase *c-Jun* expression in normal rat colon *in vivo* (Velázquez et al., 1996) suggesting that butyrate may regulate cellular proliferation by modifying transcriptional factors.

Plasma concentrations of proglucagon-derived peptides were not measured in this study but have been reported to increase with the long-term ingestion of nonabsorbed, fermentable carbohydrate and to decrease with the removal of fiber from the diet (Gee et al., 1996). These observations are consistent with reports that conditions rendering the distal bowel to greater nutrient loads produce hyperplasia (Williamson, 1982; Hanson et al., 1977; Williamson et al., 1978; Altmann and Leblond, 1970; Dowling and Booth, 1967; McDermott, 1979; Rijke et al., 1977), increased plasma enteroglucagon plasma concentrations (Morita et al., 1982; Besterman et al., 1978a, b; Bloom et al., 1972; Holst et al., 1979), and increased plasma glucagon-like peptide-1 concentrations 30 minutes after an oral glucose gavage (Reimer and McBurney, 1996). Indeed, one mechanism whereby luminal nutrition modulates small bowel adaptation may be via effects on large bowel fermentation and SCFA production and subsequent upregulation of proglucagon and ornithine decarboxylase gene expression. Studies underway in our laboratory suggest that intravenous SCFA also upregulate proglucagon gene expression in non-resected animals receiving TPN (Tappenden & McBurney, unpublished data). The exact molecular mechanism(s) whereby SCFAs initiate and moderate intestinal adaptation needs further examination although this study provides evidence that these substances may act directly on the expression of enterotrophic genes.

Table 2.1

Composition of Nutrient Solutions

Ingredient	TPN[1]	TPN+SCFA[2]
Dextrose (g/L)	204	199
Nitrogen[3] (g/L)	8	8
Lipid[4] (g/L)	26	26
NaCl (mmol/L)	60	—
Sodium acetate (mmol/L)	—	36
Sodium propionate (mmol/L)	—	15
Sodium butyrate (mmol/L)	—	9
KCl (mmol/L)	20	20
KPO_4 (mmol/L)	15	15
$C_6H_{11}O_7 \bullet 1/2Ca$ (mmol/L)	8	8
$MgSO_4$ (mmol/L)	3	3
Multivitamins[5] (mL/L)	10	10

[1] TPN represents a standard total parenteral nutrient formulation.

[2] TPN+SCFA represents a standard total parenteral nutrient formulation plus the short-chain fatty acids as indicated.

[3] 10% Travasol® (Baxter, Toronto, Ontario)

[4] 20% Intralipid® (Kabi Pharmacia, Baie D'urfe, Quebec)

[5] Multi-1000® (Sabex Inc., Boucherville, Quebec)

Table 2.2

***Effect of SCFA-Supplemented TPN on Total Ileal Weight 3 or 7 Days Following Intestinal Resection*[1]**

	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=11)	**Day 3** (n=11)	**Day 7** (n=12)	DIET	TIME
Total Wt (mg/cm)	6.4 ± 0.6	5.7 ± 0.6	7.7 ± 0.6	7.5 ± 0.6	$p < 0.05$	NS
Mucosal Wt (mg/cm)	4.0 ± 0.5	3.7 ± 0.5	4.9 ± 0.5	4.7 ± 0.5	$p < 0.05$	NS
Submucosal Wt (mg/cm)	2.5 ± 0.3	2.1 ± 0.3	2.8 ± 0.3	2.8 ± 0.3	$p < 0.05$	NS
% Mucosa	61 ± 3	64 ± 3	63 ± 3	61 ± 3	NS	NS

[1] Values are means ± SEM.

Table 2.3

***Effect of SCFA-Supplemented TPN on Ileal Protein, DNA and RNA Concentration 3 or 7 Days Following Intestinal Resection*[1]**

	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=11)	**Day 3** (n=11)	**Day 7** (n=12)	DIET	TIME
Protein (µg protein/mg ileum)	100.1 ± 4.2^{ab}	88.6 ± 4.0^{b}	109.7 ± 3.7^{a}	113.9 ± 3.8^{a}	p = 0.0001[2]	NS[2]
DNA (µg DNA/mg ileum)	1.64 ± 0.13	1.35 ± 0.14	1.72 ± 0.11	1.85 ± 0.14	$p < 0.03$	NS
RNA (µg RNA/mg ileum)	3.0 ± 0.2	3.3 ± 0.2	4.4 ± 0.3	4.5 ± 0.2	p = 0.0004	NS

[1] Values are means ± SEM.

[2] Indicates a significant interaction between diet and time. When a significant interaction exists, means within a row with different superscripts are significantly different ($p < 0.05$).

Table 2.4

Effect of SCFA-Supplemented TPN on Proglucagon and Ornithine Decarboxylase mRNAs 3 or 7 Days Following Intestinal Resection[1]

	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=11)	**Day 3** (n=11)	**Day 7** (n=12)	DIET	TIME
Proglucagon mRNA (densitometry units)	0.24 ± 0.04	0.23 ± 0.04	0.33 ± 0.04	0.32 ± 0.04	$p < 0.05$	NS
Ornithine decarboxylase mRNA (densitometry units)	0.21 ± 0.03	0.14 ± 0.03	0.27 ± 0.04	0.24 ± 0.03	$p < 0.05$	NS

[1] Values are mean ± SEM.

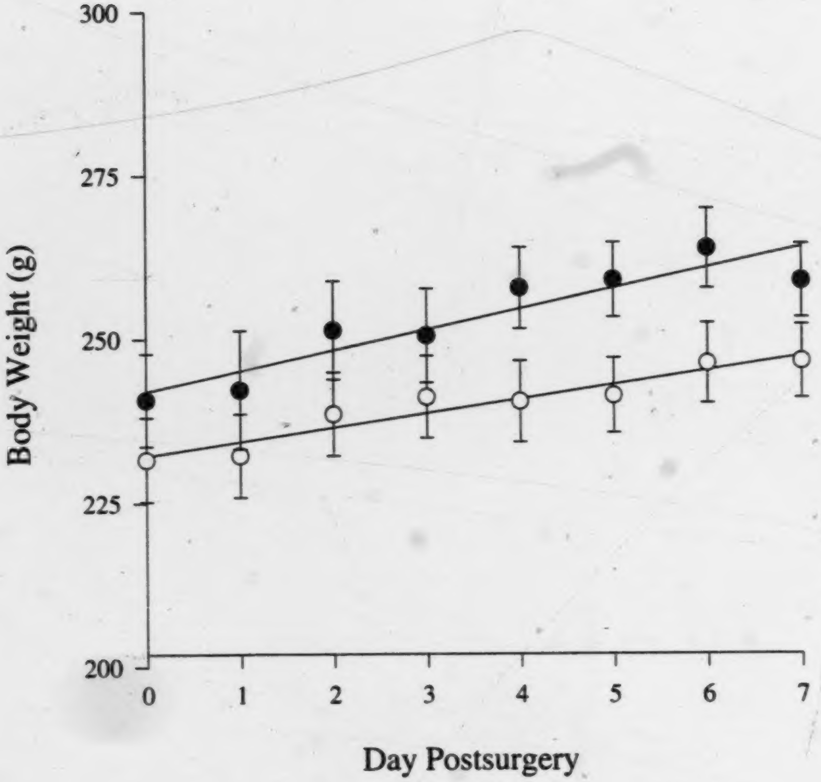

Figure 2.1 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Total Body Weight Following Intestinal Resection.

Values are means ± SEM. • TPN; ○ SCFA. As body weight did not differ between the 3 and 7 day diet groups within diets, these values were pooled (Day 0-3 TPN, n=23; Day 4-7 TPN, n=11; Day 0-3 SCFA, n=23; Day 4-7 SCFA, n=12). Total change in body weight over time did not differ between diet groups.

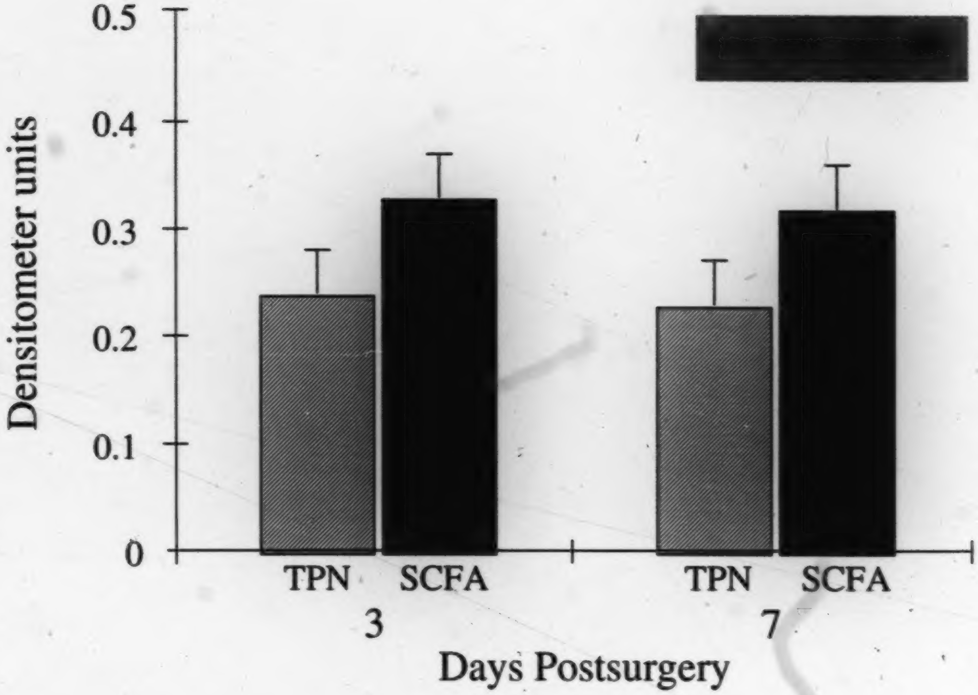

Figure 2.2 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Ileal Proglucagon mRNA Abundance Following Intestinal Resection. Values are means ± SEM. Abbreviations: TPN-3, standard total parenteral nutrition (TPN) for 3 days following surgery (n=11); TPN-7, standard TPN for 7 days following surgery (n=12); SCFA-3, short-chain fatty acid (SCFA) supplemented TPN for 3 days following surgery (n=11); SCFA-7, SCFA supplemented TPN for 7 days following surgery (n=12). The provision of SCFA-supplemented TPN for 3 and 7 days following intestinal resection increased ($p<0.05$) ileal proglucagon expression.

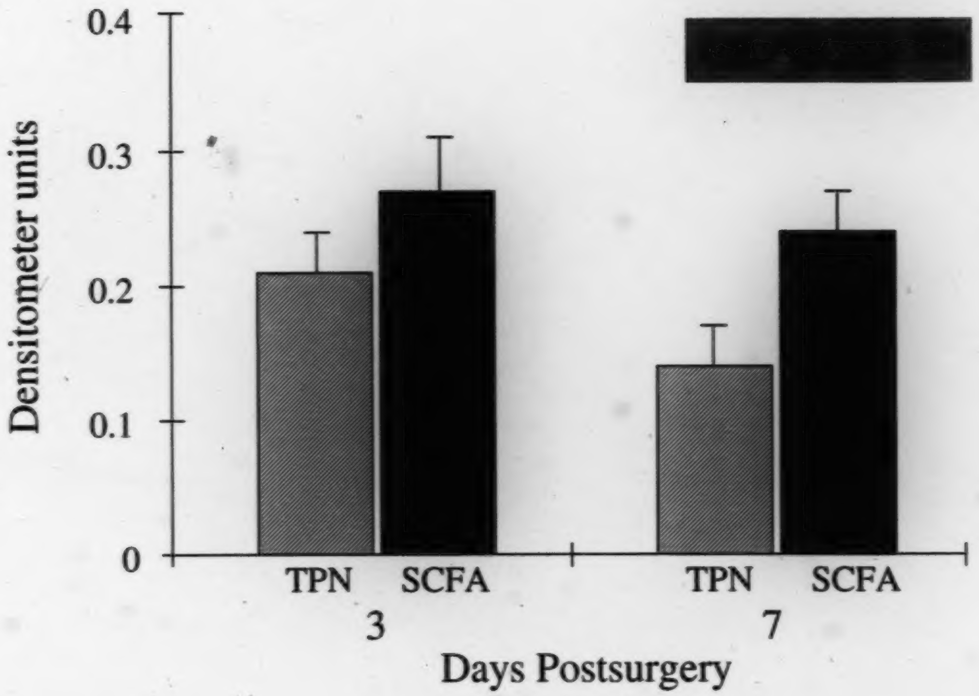

Figure 2.3 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Ileal Ornithine Decarboxylase mRNA Abundance Following Intestinal Resection.

Values are means ± SEM. Abbreviations: TPN-3, standard total parenteral nutrition (TPN) for 3 days following surgery (n=11); TPN-7, standard TPN for 7 days following surgery (n=12); SCFA-3, short-chain fatty acid (SCFA) supplemented TPN for 3 days following surgery (n=11); SCFA-7, SCFA supplemented TPN for 7 days following surgery (n=12). Ornithine decarboxylase mRNA was higher ($p<0.05$) in the SCFA-supplemented TPN groups both 3 and 7 days following surgery.

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CHAPTER 3

SHORT-CHAIN FATTY ACID-SUPPLEMENTED TOTAL PARENTERAL NUTRITION ENHANCES INTESTINAL FUNCTION FOLLOWING MASSIVE SMALL BOWEL RESECTION [4,5]

INTRODUCTION

Extensive removal of the intestine results in short bowel syndrome such that adequate intestinal adaptation and a return to enteral feedings may be unlikely. Following intestinal resection, total parenteral nutrition (TPN) is frequently administered to prevent diarrhea, dehydration, electrolyte disturbances, malabsorption and progressive malnutrition. TPN is a key therapeutic modality responsible for prolonging the lives of patients with short bowel syndrome (Purdum and Kirby, 1991; Tilson, 1980; Caniano et al., 1989; King et al., 1987). However, the use of TPN following resection is associated with intestinal atrophy and impaired functional activity (Johnson et al., 1975; Levine et al., 1974; Feldman et al., 1976; Morin et al., 1978; Castillo et al., 1991; Inoue et al., 1993; Miura et al., 1992). Byrne and associates (1995) provided preliminary evidence that nutrient absorption after massive small bowel resection can be enhanced with the combined administration of glutamine, growth hormone and a modified diet providing hope that enteral therapy may be an alternative to long-term TPN dependence. It is possible that existing TPN formulations can be modified to stimulate intestinal adaptation prior to the introduction of enteral feeding.

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[4] A version of this chapter has been accepted for publication, Tappenden KA, Thomson ABR, Wild GE, McBurney MI. Gastroenterology, in press.

[5] Awarded the American Society for Parenteral and Enteral Nutrition 1996 Dietitian Research Award at the 20th Clinical Congress in Washington, D.C. and published, in part, in abstract form, JPEN 1996;20:60.

TPN supplemented with short-chain fatty acids (SCFAs), the byproduct of dietary fiber fermentation in the colon, has been shown to prevent TPN-associated mucosal atrophy and thus facilitate morphological adaptation to small bowel resection in rats (Koruda et al., 1988; Chapter 2). The effect of SCFAs on the functional aspects of intestinal adaptation remains unknown but it is reasonable to assume that SCFA-induced intestinal hypertrophy requires a concomitant increase in transport of other nutrients such as glucose, amino acids and lipids. The hypothesis that SCFA supplementation of TPN results in increased glucose transport and mRNA abundance of intestinal glucose transporters was tested in the present study.

MATERIALS AND METHODS

Animals

Forty-eight adult male Sprague-Dawley rats (weighing 249±10 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet (Marsman and McBurney, 1995) and drinking water. The elemental diet was given to minimize the effect of residual dietary fiber fermentation and therefore decrease SCFA production in the gastrointestinal tract prior to the onset of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care.

Surgical Model

Prior to surgery, animals underwent a 12 hour food restriction, were weighed and anesthetized with halothane. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (1981). Subsequently, all animals were subjected to an 80% jejunoileal resection, leaving 1-cm of jejunum distal to the ligament of Treitz and 15-cm of ileum proximal to the ileocecal junction. The 15-cm intestinal segment proximal to the ileocecal valve was measured using a 15-cm length of 3-0 silk suture placed along the anti-mesenteric border of the gently stretched small intestine. Thus, all animals were left with an equivalent length of distal ileum and 1-cm of proximal jejunum. Bowel continuity was restored by an end-to-end jejunoileal anastomosis with interrupted 6-0 silk sutures. The abdomen was closed with interrupted 3-0 dexon sutures. Sterile instruments and aseptic technique were used at all times.

Postoperative Care and Nutrient Solutions

Postoperatively, animals were randomly assigned to receive either standard TPN or an isoenergetic, isonitrogenous TPN supplemented with SCFAs. Within these two diet groups, animals were further randomized to receive the parenteral solutions for either 3 or 7 days. The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain optimal sterile conditions and were filter sterilized before infusion (0.22μm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemicals, St. Louis, MO) in the concentrations used by Koruda and associates (1988) and in the molar proportions found physiologically in the colon (Cummings, 1984). Weak anions, such as SCFAs, exert a stabilizing influence on anionic lipid stabilizers and use of the more stable calcium gluconate, instead of calcium chloride further improved the admixture stability (Driscoll, 1995). All solutions were compounded (Keck-Jones, 1994) and handled according to guidelines that ensured safe administration (Driscoll, 1994). The diets were infused daily to provide 52 kcal and 425 mg nitrogen with a nonprotein kilocalorie-to-nitrogen ratio of 116. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue Preparation

On the third (TPN-3, n=12; TPN+SCFA-3, n=12) and seventh (TPN-7, n=12; TPN+SCFA-7, n=12) postoperative days, animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of it's mesenteric fat, rinsed in ice-cold saline and weighed. A 2-cm section of tissue, located 2-cm distal to the anastomosis, was removed, snap frozen in liquid nitrogen and stored at -70°C for subsequent RNA isolation and quantification (Chomzynski and Sacchi, 1987). A 1-cm segment (located 1-cm proximal to the ileocecal valve) was stored at -70°C until assayed for DNA (Prasad et al., 1972) and protein (Lowry et al., 1951). Finally, a 10-cm segment of ileum, located 4-cm distal to the anastomosis was removed for intestinal glucose uptake studies and mucosal wet weight determination.

Measurement of Transport Kinetics

Determination of transport kinetics was done as previously described (Thomson and Rajotte, 1983). Briefly, the 10-cm segment of ileum removed from each animal was opened along it's mesenteric border and the mucosal surface was carefully washed with cold saline to remove visible mucus and debris. Pieces of intestine (1-cm^2) were cut out and the tissue was mounted as flat sheets in incubation chambers containing oxygenated Kreb's bicarbonate buffer (pH 7.4) at 37°C. Tissue discs were preincubated in this buffer for 15 minutes to allow equilibration at this temperature. After preincubation, the chambers were transferred to other beakers containing [^{3}H]-inulin and various [^{14}C]-probe molecules in oxygenated Krebs bicarbonate (pH 7.4 and 37°C). The concentration of solutes was: *D*-glucose 4, 8, 16, 32, or 64 m*M*, and *L*-glucose 16 m*M*. The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars, and the stirring rates were precisely adjusted by means of a strobe light. A stirring rate of 600 rpm was selected to achieve low effective resistance of the intestinal unstirred water (Thomson and Dietschy, 1980). The experiment was terminated by removing the chambers and quickly rinsing the tissue in cold saline for approximately 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch. For all probes the tissue was dried overnight in an oven at 55°C . The dry weight of the tissue was determined, the sample was saponified with 0.75 *N* NaOH, scintillation fluid was added (Beckman Ready Solv HP, Beckman Canada, Mississauga, ON), and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes. The mucosal weight was determined following scraping of the intestine from adjacent samples not used for uptake studies. The weight of the mucosa in the samples used to measure uptake was determined by multiplying the dry weight of the intestinal sample by the percentage of the intestinal wall comprised of mucosa. As the proportion of mucosa in the total ileum did not differ between groups, the uptake of nutrients was expressed as nmol•100mg ileum^{-1}•minute^{-1}.

Northern Analysis

RNA was electrophoresed on denaturing 1% (w/v) agarose/0.66 *M* formaldehyde gels and transferred to MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA) by capillary diffusion. The integrity and relative amounts of RNA were assessed by ultraviolet light visualization of ethidium bromide-stained RNA. For northern analysis with the GLUT2, GLUT5, SGLT-1 and sucrase-isomaltase probes, membranes were prehybridized for 2 hr at 50°C in a medium containing 60% (v/v) formamide, 1 x SSPE (0.18 *M* NaCl, 0.01 *M* sodium phosphate at pH 7.4, 1 m*M* EDTA), 0.5% (w/v) blotto, 10% (w/v) dextran sulfate, 1% (w/v) sodium dodecyl sulfate and 500 µg/mL salmon testes DNA. Following prehybridization, hybridization was carried out for 16-18 hours at 50°C in fresh buffer containing [^{32}P]-labeled probes (10^6 cpm/mL). Membranes were then rinsed briefly in 2 x SSC (1 x SSC is 0.15 *M* NaCl, 0.015 *M* sodium citrate at pH 7.0), washed for 15 minutes at room temperature in 2 x SSC containing 0.1% sodium dodecyl sulfate, washed at 70°C in 0.2 x SSC containing 1% sodium dodecyl sulfate and rinsed briefly in 0.2 x SSC. The washed membranes were exposed to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (DuPont Canada, Mississauga, ON) at -70°C.

The membranes hybridized with the Na^+, K^+-ATPase α_1 and β_1 isoform subunit probes were prehybridized (7% SDS, 50% formamide, 5 x SSC, 2% blocking reagent [Boehringer Mannheim, Laval, PQ], 50m*M* sodium phosphate @ pH 7.0, 0.1% N-laurylsarcosine) at 42°C for 4 hours. Hybridization was carried out for 12 to 16 hours at 42°C in an identical volume of fresh hybridization solution containing 50 ng/mL of digoxigenin-labelled probe (Feinberg and Volgenstein, 1983). Membranes were then washed 3 x 10 minutes at room temperature in 2 x SSC (3 m*M* NaCl and 300 m*M* sodium citrate, pH 7.0) and 3 x 15 minutes at 68°C in 0.1 x SSC. The washed membranes were equilibrated in maleate buffer (100 m*M* NaCl, 100 m*M* maleic acid, pH 7.5) and blocked for 2 to 3 hours in 1% blocking reagent. The membranes were then incubated with anti-digoxigenin-alkaline phosphatase conjugated antibody (1:100 000 dilution) for 30 minutes. Membranes were rewashed in maleate buffer containing 0.3% Tween for 4 x 15 minutes at room temperature and then in 100 mM Tris-HCl/100 mM NaCl (pH 9.5) buffer for 2 minutes at room temperature to activate the alkaline phosphatase. The bound probe was detected using Lumigen PPD (Boehringer Mannheim) as the chemiluminescent substrate. The membranes were exposed overnight to X-OMAT-AR (Eastman Kodak, Rochester, NY) film at room temperature.

Relative mRNA concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, ON). The 28S ribosomal units were quantified from photographs of the ethidium bromide stained membranes prior to hybridization. These values were used to compensate for any loading discrepancies, variations in RNA integrity or nonspecific changes in RNA abundance.

Nutrient Transporter Probes

A radiolabelled GLUT2 antisense riboprobe was generated from Xba I-linearized plasmid DNA [pGEM4Z-HTL-3] (Fukumoto et al., 1989) and T7 RNA polymerase. A GLUT5 cDNA insert from plasmid pUC13-phJHT-5 (Kayano et al., 1990) was subcloned into the BAM HI and Eco RI sites of gPEM3 plasmid and antisense RNA was synthesized using Ban HI-linearized plasmid and T7 RNA polymerase. The GLUT2 and GLUT5 plasmids were kindly donated by Dr. G.I. Bell (Howard Hughes Medical Institute, University of Chicago). The SGLT-1 cDNA probe was generated from the EcoR1 site of the pGEM-4Z vector which was a generous gift from Dr. N.O. Davidson at the University of Chicago. The cDNA probes encoding the α_1 and β_1 Na^+,K^+-ATPase subunit isoforms were obtained from Dr. J Lingrel (University of Cincinnati). Northern hybridization was carried out with 300 bp EcoR1 - PstI α_1 and NcoI - StuI β_1 fragments. Dr. Peter G. Traber (University of Pennsylvania) kindly supplied the sucrase-isomaltase cDNA which was generated from the pGEM-4Z vector at the EcoR1 site.

Statistics

Differences between treatments were determined using a randomized block ANOVA. Sources of variation were blocks (surgery day=12), diet (d=2), time (t=2) and diet interacted with time. Comparisons within the diet by time interaction were made using student's t-test. The effect of diet on glucose uptake was determined using a repeated measures ANOVA. Statistical significance was defined as $p \leq 0.05$. Computations were done using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC). All data are presented as mean±SEM.

RESULTS

Body Weight

All animals received continuous nutrient infusion throughout the study period and increased in weight an average of 2.4 g/day. Total change in body weight did not differ between diet groups (Day 3, +10 ± 5 g; Day 7, +17 ± 6 g). All animals grew at a rate comparable to nonsurgical, *ad libitum* chow fed controls (data not shown) indicating the adequacy of the nutritional support provided.

In Vitro Glucose Uptake

Ileal uptake of 4-64 m*M* *D*-glucose was increased (P=0.003) in the TPN+SCFA group both 3 and 7 days following intestinal resection (Figure 3.1). The mean values of the maximal transport rates (V_{MAX}) were significantly increased ($p<0.05$) in the TPN+SCFA group at both time points when expressed as nmol•100mg ileum^{-1}•minute^{-1} (Table 3.1). There were no differences in the estimated values for the apparent Michaelis affinity constant (K_M) (Table 3.1). The apparent passive permeability coefficient, estimated with *L*-glucose, did not differ among groups (TPN, 119±12 nmol•100mg ileum^{-1}•minute^{-1}; TPN+SCFA, 131±10 nmol•100mg ileum^{-1}•minute^{-1}).

Northern Blot Analysis of GLUT2 mRNA

Northern analysis confirmed the presence of the 3.8 kb GLUT2 mRNA species in the ileal total cellular RNA (Figure 3.2). Ileal GLUT2 mRNA abundance was significantly higher (p=0.007) in the TPN+SCFA group 3 days following surgery (Table 3.2).

Northern Blot Analysis of GLUT5 mRNA

The GLUT5 riboprobe hybridized the 3.2 kb transcript in the ileal total cellular RNA (Figure 3.3). GLUT5 mRNA abundance did not differ between groups (Table 3.2).

Northern Blot Analysis of SGLT-1 mRNA

Northern analysis confirmed the presence of the 4.8 kb SGLT-1 transcript (Figure 3.4). The abundance of SGLT-1 mRNA tended to be increased (p=0.1) in the TPN+SCFA group at both time points, however this trend was not statistically significant (Table 3.2).

Northern Blot Analysis of Sucrase-Isomaltase mRNA

The sucrase isomaltase cDNA probe detected the 6.0 kb transcript within the total cellular RNA (Figure 3.5). The sucrase-isomaltase mRNA abundance did not differ between treatment groups (Table 3.2).

Northern Blot Analysis of the α_1 and β_1 Na^+, K^+-ATPase subunit isoform mRNA

Hybridization with the α_1 cDNA probe disclosed a single 3.7 kb transcript (Figure 3.6). The level of α_1 Na^+,K^+-ATPase subunit isoform mRNA abundance was significantly lower ($p<0.05$) in the 3 day TPN+SCFA group following surgery (Table 3.3). The β_1 Na^+,K^+-ATPase isoform subunit cDNA probe hybridized to the 2.7 kb transcript (Figure 3.7). The β_1 Na^+,K^+-ATPase isoform subunit mRNA abundance was lower ($p<0.05$) in the 3 day TPN+SCFA group (Table 3.2).

DISCUSSION

TPN alimentation provides an ideal model with which to study the role of specific nutrients in intestinal adaptation following resection while providing adequate nutrition support. Massive small bowel resection in rats reliably produces a syndrome characterized by massive prolonged diarrhea, malabsorption of enteral nutrients, negative nitrogen balance and loss of body weight (Roth et al., 1995). Consequently, the use of oral diets in resected animals results in malabsorption and confounds the effects of specific nutrients with malnutrition. In addition, the use of parenteral support allows one to examine the effect of specific nutrients (i.e. SCFAs) versus nonspecific effects of luminal stimulation on intestinal adaptation. Finally, the identification of nutrients which can be administered systemically to stimulate intestinal function is of significant clinical relevance.

The nutrient solutions were designed and administered to meet energy and protein requirements for a 250 g rat. Energy and nitrogen intakes were elevated over those previously published (Koruda et al., 1988) and reductions in body weight are frequently reported using this highly catabolic model (Al-Mukhtar et al., 1983; Koruda et al., 1986, 1988; Aghdassi et al., 1994). We view the gain in body weight during the hypermetabolic period following surgery (Cuthbertson, 1936; Vinnars et al., 1969; Elwyn, 1980) to be a significant accomplishment. Despite many studies identifying SCFAs as gut-specific fuels (reviewed by Cummings, 1995), the metabolism of systemically administered SCFAs by other organs and tissues is likely uninhibited and the amount being used by the gastrointestinal tract as a fuel is unknown.

SCFAs enhance functional parameters of intestinal adaptation, as shown by increased ileal uptakes of *D*-glucose, at days 3 and 7. The greater *D*-glucose uptakes observed with SCFAs reflect an increase in the V_{MAX} of *D*-glucose which is suggestive of an increase in the number of nutrient transporters per enterocyte. This observation agrees with reports that changes in sugar and amino acid uptakes result from alterations in V_{MAX} (Karasov and Diamond, 1987).

In a TPN model, nutrients available to enterocytes are at the basolateral membrane. The mRNA abundance of the basolateral facilitative glucose transporter, GLUT2, increased in the 3 day TPN+SCFA group whereas that of the brush border facilitative fructose transporter, GLUT5, did not differ among groups. Expression of the brush border Na^+/glucose cotransporter, SGLT-1, was not significantly changed with SCFA supplementation but a role for upregulation of this transporter in SCFA-induced adaptation cannot be excluded. Regulation of SGLT-1 is believed to be translational or post-translational as changes in mRNA levels dramatically underestimate changes in co-transporter number and activity (Lescale-Matys et al., 1993). Furthermore, the adaptive response of the brush border and basolateral membrane domains of the enterocyte seem to occur such that neither domain becomes a significant rate limiting step (Cheeseman, 1992). Since transporter protein abundance was not measured in this study we cannot attribute the increase in functional glucose transport observed with SCFA-supplementation to any one transporter but the apparent dissociation between SGLT-1 mRNA and transport activity is not unprecedented. We are not aware of conclusive evidence suggesting similar regulation of the other transporters measured. The brush border GLUT5 fructose transporter appears to be transcriptionally regulated as changes in intestinal GLUT5 expression correlate with reported alterations in intestinal absorption of fructose (Castello et al., 1995). Likewise, a recent report by Rencurel and associates

(1996) suggests that GLUT2 is also transcriptionally regulated. It is possible that sucrase-isomaltase mRNA levels remain unchanged yet activity is upregulated due to enhanced biosynthesis, alterations in protein half-life or recruitment of intracellular proteins to their functional site. Hoffman and Chang (1991) determined that a large proportion of jejunal sucrase-isomaltase mRNA was associated with membrane-bound polyribosomes, accounting for the 3-4 fold difference in enzyme activity along the longitudinal axis of the gut in the absence of any difference in mRNA levels. Conclusions regarding the effect of SCFA-supplemented TPN on sucrase-isomaltase activity following massive small bowel resection await further study.

The Na^+,K^+-ATPase is responsible for the maintenance of the Na^+ and K^+ electrochemical gradients across the cell membrane in virtually all mammalian cells (Horisberger et al., 1991). In the enterocyte, the restriction of Na^+, K^+-ATPase to the basolateral membrane facilitates the vectorial transport of Na^+ and K^+ and the maintenance of the transcellular Na^+ gradient required for the Na^+-dependent nutrient cotransporters that are restricted to the brush-border membrane (Schultz and Hudson, 1991; Thorens, 1993; Wright, 1993). Given the central role of Na^+, K^+-ATPase in intestinal electrolytes and nutrient absorptive process, the implications of reduced Na^+, K^+-ATPase mRNA abundance in the 3 day SCFA-supplemented group should not be overlooked. We did not measure fluid and electrolyte transport in this model, but Bowling and associates (1993) reported that colonic fluid secretion induced by a standard polymeric enteral diet was reduced with colonic SCFA infusion. Although Na^+, K^+-ATPase activity was not measured, movement of sodium, chloride, and potassium ions was similar to that of water in all stages of the study. Similarly, water and sodium absorption from the rectum of 24 patients with acute watery diarrhea was normalized with luminal SCFAs (Ramakrishna and Mathan, 1993). In the current study, animals were fed intravenously and did not receive any enteral nutrients to be transported across the brush border membrane. The lower Na^+,K^+-ATPase mRNA abundance within the 3 day SCFA group may reflect the lack of sodium dependent brush border transport *per se* since increased basolateral transport of glucose, via the sodium independent GLUT2 would not require Na^+, K^+-ATPase. Similarly, basolateral amino acid transport is not completely blocked with ouabain (Chandler et al., 1993; Ma et al., 1994). Finally, the lower Na^+, K^+-ATPase mRNA abundance within the 3 day SCFA supplemented TPN group may simply represent a decrease as a relative proportion of the total genes undergoing transcription in the groups. The state of enterocyte differentiation may be a key factor in determining Na^+, K^+-ATPase expression (Giannella et al., 1993; Wild and Murray, 1992) and the physical properties of the enterocyte plasma membrane as well as membrane-cytoskeleton interactions may also influence Na^+, K^+-ATPase activity and absorptive function (Madsen et al., 1992; Molitoris et al., 1992). Further study is needed to assess the functional effect of SCFA-supplemented TPN following massive small bowel resection on Na^+, K^+-ATPase activity.

The manner in which SCFAs influence intestinal mucosal proliferation is not addressed in the current study. SCFAs, particularly acetate, increase intestinal blood flow which may enhance mucosal proliferation (Dowling and Booth, 1967). Intravenous infusion of SCFAs increase pancreatic secretions (Harada and Kato, 1982) and although not extensively studied, are suspected to stimulate enterohormone secretions (Koruda et al., 1988). Proglucagon and ornithine decarboxylase mRNA are more abundant in ileum from TPN supported animals receiving SCFAs following intestinal resection (Chapter 2). Proglucagon derived peptides have been identified as potential mediators in intestinal adaptation (Rountree et al., 1992; Taylor et al., 1990), however it remains uncertain whether transported substrates themselves serve as the regulatory signals, or if intermediate signals such as hormones are required. This study is unique in that it suggests a specific nutrient (i.e. SCFAs) may modulate gene expression and transport capacity of other nutrients (i.e. glucose). It remains to be elucidated whether SCFAs exert their effect through stimulation of gastrointestinal growth factors or by direct action on the enterocyte.

In summary, this study reveals that SCFA supplementation of TPN enhances morphological and functional aspects of adaptation in rats subsequent to small bowel resection. It may be possible to modify current TPN formulations to stimulate nutrient transport and facilitate the introduction of enteral feeding in patients with short bowel syndrome.

Table 3.1

Effect of SCFA-Supplemented TPN on the Kinetic Characteristics of D-Glucose Uptake 3 or 7 Days Following Intestinal Resection[1]

	TPN		TPN+SCFA		DIET	TIME
	Day 3 (n=12)	**Day 7** (n=12)	**Day 3** (n=12)	**Day 7** (n=12)		
V_{MAX}	844 ± 189	690 ± 223	1333 ± 186	1021 ± 189	P < 0.05	NS
KM	33 ± 10	57 ± 13	50 ± 10	33 ± 10	NS	NS

[1] Values are mean ± SEM.

Table 3.2

Effect of SCFA-Supplemented TPN on the Expression of Various Enterogenes 3 or 7 Days Following Intestinal Resection[1]

densitometry units	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=12)	**Day 3** (n=12)	**Day 7** (n=12)	DIET	TIME
GLUT2 mRNA	3.7 ± 1.6^{a}	3.2 ± 1.5^{a}	10.1 ± 1.6^{b}	2.8 ± 1.4^{a}	$p < 0.05$[2]	NS[2]
GLUT5 mRNA	3.9 ± 0.6	3.7 ± 0.6	4.2 ± 0.6	4.1 ± 0.6	NS	NS
SGLT-1 mRNA	6.3 ± 2.9	8.6 ± 2.7	11.8 ± 2.9	10.5 ± 3.0	$p = 0.10$	NS
Sucrase-Isomaltase mRNA	2.2 ± 0.4	2.6 ± 0.4	2.3 ± 0.4	2.1 ± 0.4	NS	NS
Na^{+},K^{+}-ATPase α_1 mRNA	8.2 ± 0.8^{a}	7.4 ± 0.8^{a}	4.3 ± 0.9^{b}	7.7 ± 0.8^{a}	$p = 0.04$[2]	NS[2]
Na^{+},K^{+}-ATPase β_1 mRNA	8.3 ± 1.2^{ab}	9.7 ± 1.2^{a}	5.5 ± 1.2^{b}	9.7 ± 1.2^{a}	NS[2]	$p = 0.03$[2]

[1] Values are mean ± SEM.

[2] Indicates a significant interaction between diet and time. When a significant interaction exists, means within a row with different superscripts are significantly different ($p<0.05$).

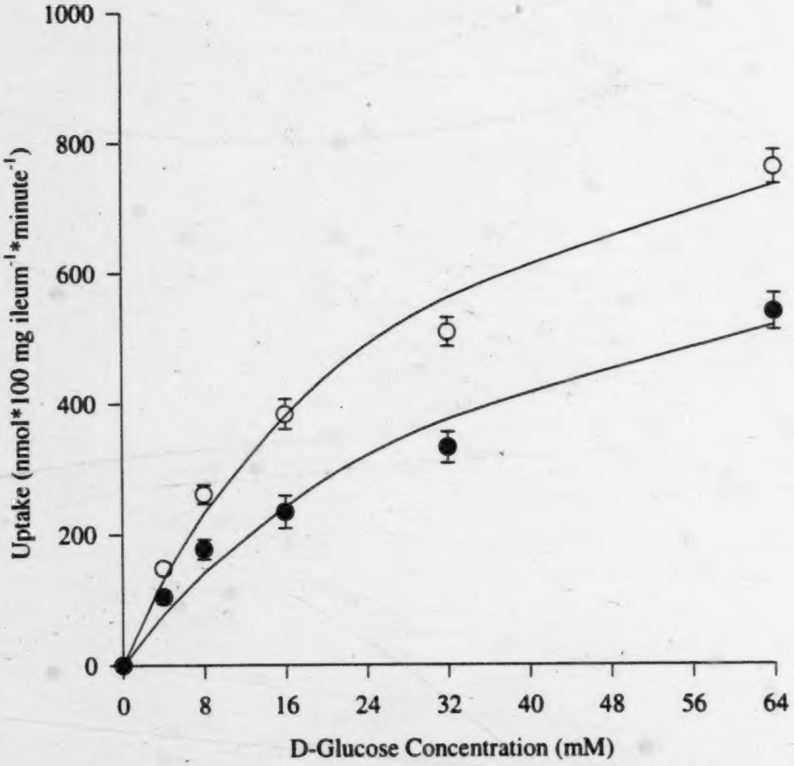

Figure 3.1 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on Ileal Glucose Uptake Following Intestinal Resection.

● TPN; ○ SCFA. Values are means ± SEM. Values are pooled within diets as uptakes did not differ between day 3 and 7. The SCFA supplementation of TPN (TPN+SCFA-3, n=12; TPN+SCFA-7, n=12) increased ($p=0.003$) ileal glucose uptake when compared to the unsupplemented TPN (TPN-3, n=12; TPN-7, n=12) at all D-glucose concentrations.

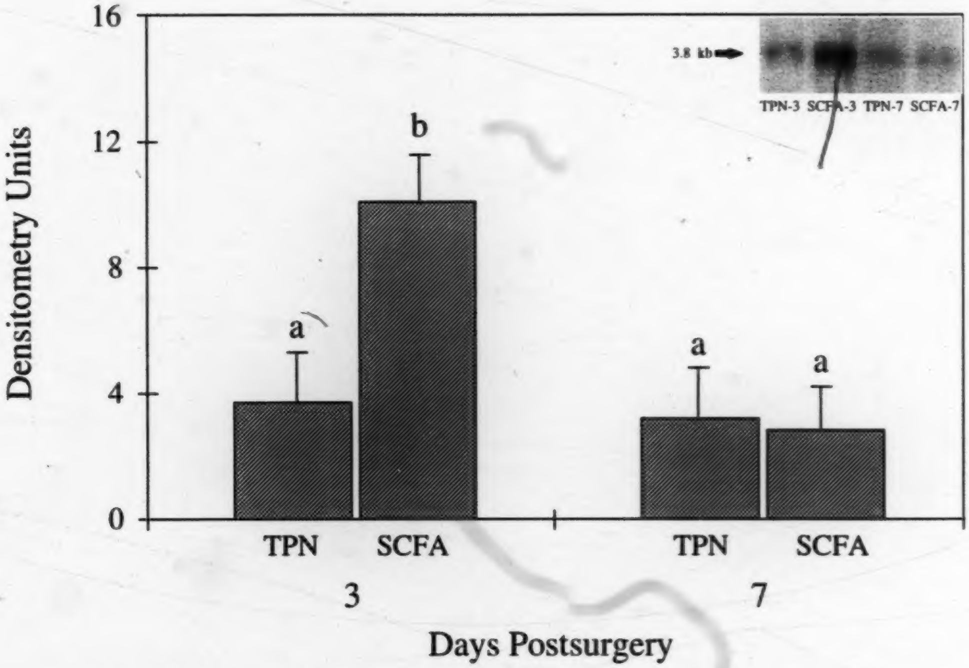

Figure 3.2 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on GLUT2 mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 (TPN+SCFA-3, n=12) days resulted in significantly greater abundance of GLUT2 mRNA ($p<0.007$) when compared to all other groups (TPN-3, n=12; TPN-7, n=12; TPN+SCFA-7, n=12). Bars that do not share a common superscript are significantly different as determined by a two-way ANOVA and student's t-test.

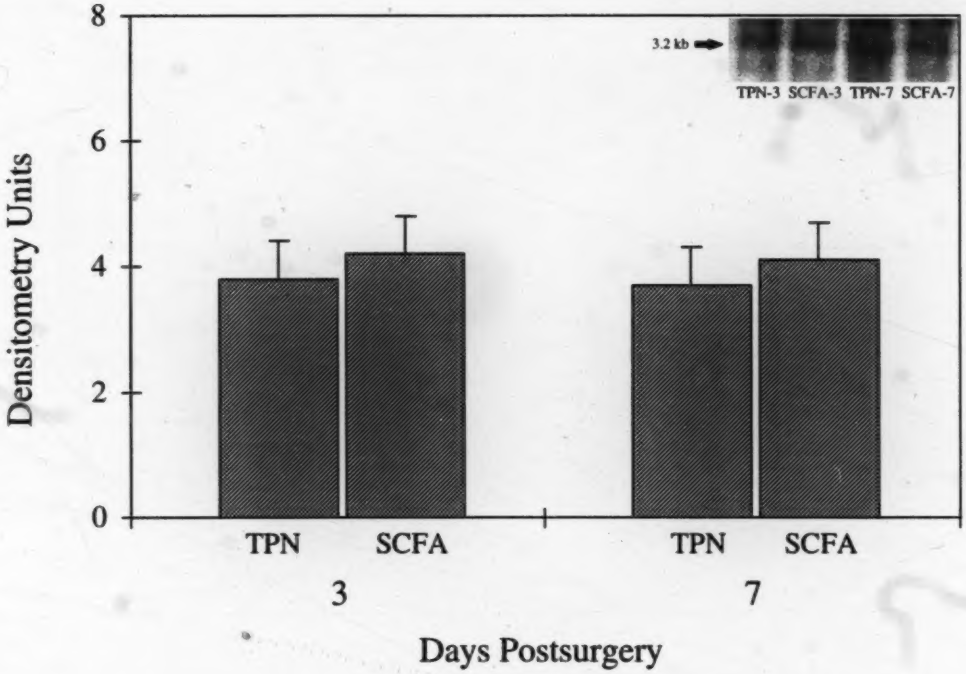

Figure 3.3 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on GLUT5 mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 (TPN+SCFA-3, n=12) and 7 (TPN+SCFA-7, n=12) days had no effect on GLUT5 mRNA abundance when compared to the unsupplemented TPN groups at both time points (TPN-3, n=12; TPN-7, n=12).

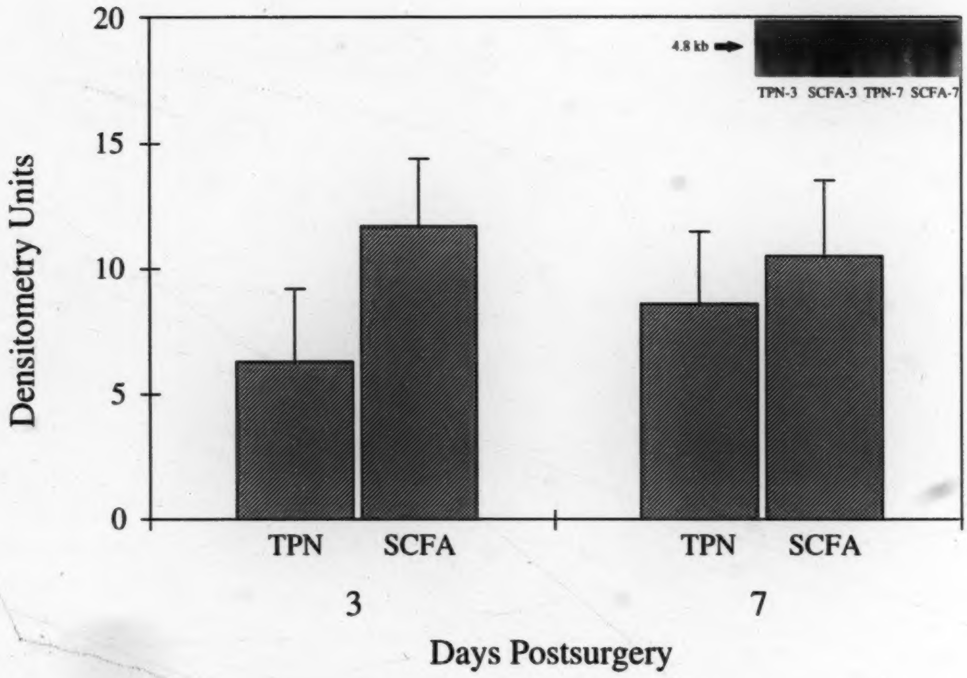

Figure 3.4 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on SGLT-1 mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 (TPN+SCFA-3, n=12) and 7 (TPN+SCFA-7, n=12) days tended to increase SGLT-1 mRNA abundance (p=0.1) when compared to the unsupplemented TPN groups at both time points (TPN-3, n=12; TPN-7, n=12).

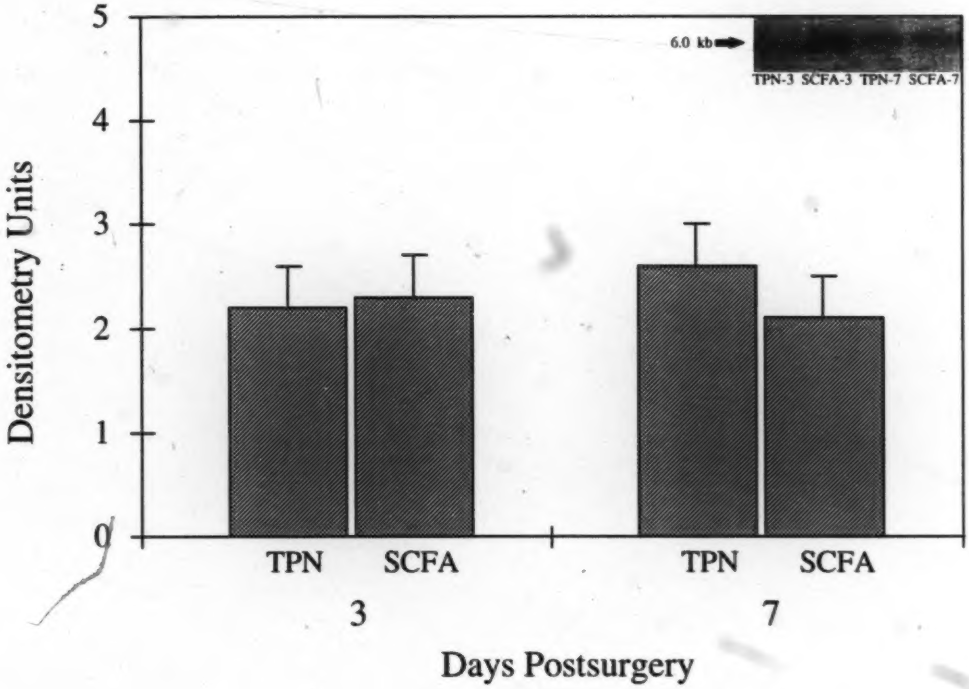

Figure 3.5 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on Sucrase Isomaltase mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 (TPN+SCFA-3, n=12) and 7 (TPN+SCFA-7, n=12) days did not alter sucrase-isomaltase mRNA abundance when compared to the unsupplemented TPN groups at both time points (TPN-3, n=12; TPN-7, n=12).

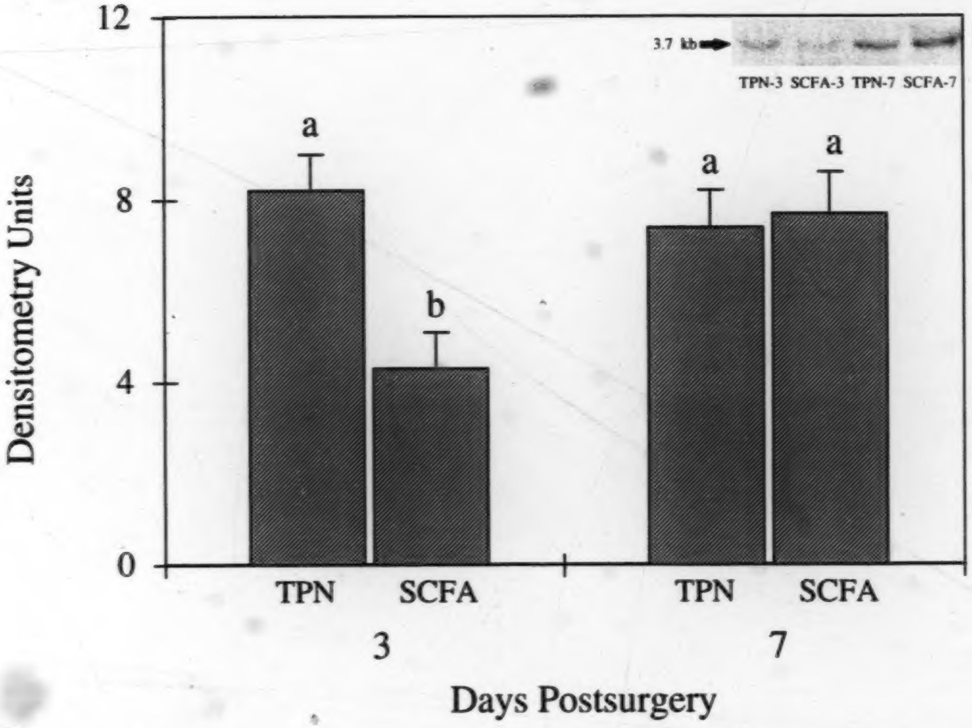

Figure 3.6 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on Na$^+$, K$^+$-ATPase α_1 Isoform Subunit mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 days (TPN+SCFA-3, n=12) resulted in significantly less ($p<0.05$) Na$^+$,K$^+$-ATPase α_1 isoform subunit mRNA when compared to all other groups (TPN-3, n=12; TPN-7, n=12; TPN+SCFA-7, n=12). Bars that do not share a common superscript are significantly different as determined by a two-way ANOVA and student's t-test.

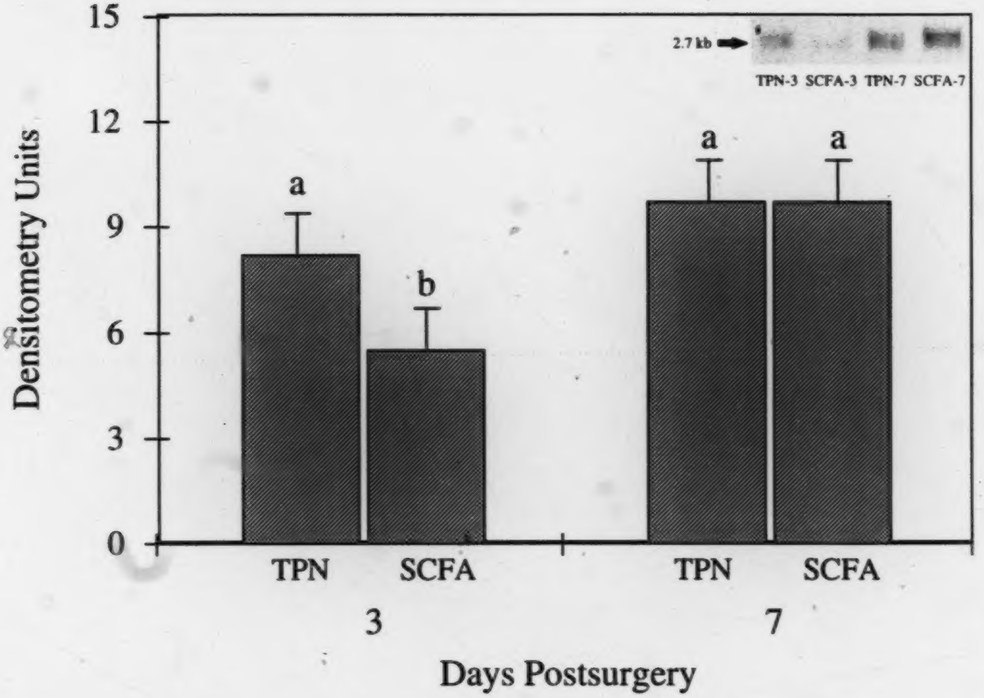

Figure 3.7 The Effect of Short-Chain Fatty Acid Supplementation of Total Parenteral Nutrition on Na$^+$,K$^+$-ATPase β_1 Isoform Subunit mRNA Abundance 3 or 7 Days Following Intestinal Resection.

Values are means ± SEM. The provision of SCFA-supplemented TPN for 3 days (TPN+SCFA-3, n=12) resulted in significantly less ($p<0.05$) Na$^+$,K$^+$-ATPase β_1 isoform subunit mRNA when compared to all other groups (TPN-3, n=12; TPN-7, n=12; TPN+SCFA-7, n=12). Bars that do not share a common superscript are significantly different as determined by a two-way ANOVA and student's t-test.

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CHAPTER 4

SHORT-CHAIN FATTY ACID-SUPPLEMENTED TOTAL PARENTERAL NUTRITION IMPROVES NONSPECIFIC IMMUNITY AFTER INTESTINAL RESECTION IN RATS [6,7]

INTRODUCTION

The use of total parenteral nutrition (TPN) in the treatment of patients with malabsorption and malnutrition is associated with immunosuppression (Deitch et al., 1991; Moore et al., 1989, 1992) and intestinal atrophy (Koruda et al., 1988). These changes are reflected clinically in an increased incidence of infections in TPN- versus enterally-fed patients (Kudsk et al., 1992; Mainous et al., 1991; McGeer et al., 1990; Cleary and Pickering, 1983) which may relate to changes in intestinal mucosal barrier and the responsiveness of the gut-associated immune system. The mechanism for the increased susceptibility to infection and immunosuppression associated with TPN is not known.

The nonspecific immune system is the first line of defense against invading pathogens. Cells involved in nonspecific immunity include macrophages, neutrophils and natural killer (NK) cells. During TPN, reduced macrophage and neutrophil phagocytic (Cleary and Pickering, 1983) and chemotactic activity (Fraser et al., 1983; Hawley and Gordon, 1976) have been reported. TPN is associated with decreased NK cell activation and lower cytotoxic activity of lymphokine-activated killer cells (Monson et al., 1988; Pollack et al.,

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1991). Impairment of nonspecific defenses is clinically manifested as frequent septic and nonseptic complications (Moore et al., 1989).

TPN also has an adverse effect on specific or cell-mediated immunity (Gogos et al., 1990; Kinsella et al., 1990; Sedman et al., 1991; Shou et al., 1994). Cell-mediated immunity is the acquired antigen specific system composed of T and B lymphocytes. Cell-mediated immunity is critical in combating invading pathogens. Different lymphocyte populations change during TPN and result in a net effect of a reduction in CD4:CD8 (T helper cell to T suppressor cell) ratio (Gogos et al., 1990). *In vitro,* proliferative responses of lymphocytes from both peripheral blood and spleen are reportedly depressed during TPN (Mainous et al., 1990; Shou et al., 1994) and are indicative of poor patient prognosis (Wolfe et al., 1982). Thus, immunosuppression occurring during TPN results in an inability to control invading pathogens through impairment of both cell-mediated and nonspecific immune functions. This observation has prompted the supplementation of specific nutrients (i.e. long and medium chained fatty acids, glutamine) to TPN formulations in an attempt to improve immune function in patients receiving such support (Gottschlich, 1992).

The short chain fatty acids (SCFAs; propionate, acetate and butyrate) have beneficial effects on the gastrointestinal tract. SCFAs are a rapidly absorbed energy source (Birkhan and Border, 1981; Settle, 1988; Yang et al., 1970) and stimulate mucosal growth (Koruda et al., 1990; Kripke et al., 1989; Sakata and Englehardt, 1983). SCFAs have been shown to prevent TPN-associated atrophy (Koruda et al., 1988) however, the effects of SCFAs on immune function are not known. SCFAs are important in maintaining the integrity of the mucosal barrier (Elsen and Bistrian, 1991; Koruda et al., 1988, 1990), therefore, it is logical to hypothesize that the immune status of recipients of TPN will improve when SCFAs are included in the parenteral solution. The epithelial cells of the gastrointestinal tract turnover rapidly, therefore intervention that result in changes in gastrointestinal enzymes and hormones may be seen as early as 3 days postsurgery. Clinically, the average length of time patients are on TPN is 7 days. Therefore 3 and 7 days were selected as time points to determine the effects of SCFA supplementation of standard TPN on components of the specific and nonspecific immune system following massive small bowel resection.

MATERIALS AND METHODS

Materials

Sodium ^{51}chromate and [^{3}H]-thymidine were obtained from Amersham Canada, Oakville, ON, Canada. RPMI 1640 culture media was purchased from Fisher Scientific, Edmonton, AB, Canada and antibiotic-antimytotic from Gibco, Burlington, ON, Canada. All other culture ingredients, Concanavalin A (Con A) and Phorbol Myristate Acetate (PMA) and Ecolite® scintillation fluid were obtained from ICN, Montreal, PQ, Canada. Bovine serum albumin (Fraction V), trypan blue, Ionomycin (Iono), EDTA and SCFA (sodium salts) were purchased from Sigma Chemical Co., St. Louis, MO. Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG was purchased from Organon Teknika Inc., Scarborough, ON, Canada. All monoclonal antibodies and natural killer sensitive YAC-1 cells were provided by Dr. A. Rabinovitch (University of Alberta, Edmonton, AB, Canada) with the exception of 3.2.3 which was purchased from Cedarlane (Hornby, ON, Canada). Membrane filters (0.22 µmol millipore) were obtained from Millipore Corporation, Bedford, MA. Harvard infusion pumps were obtained from Harvard Apparatus, Wellesley, MA. Swivels were obtained from Instech Laboratories, Plymouth Meeting, PA.

Animals and Surgical Procedures

All procedures received ethical approval from the Faculty Animal Policy and Care Committee of the University of Alberta and are consistent with the guidelines of the Canadian Council on Animal Care.

Fifty-nine adult male Sprague-Dawley rats (237 ± 3 g) were obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Alberta, Canada) and acclimatized to the laboratory for 4 days in individual metabolic cages with 12 hour light/dark exposure. Four days prior to surgery, rats were provided *ad libitum* with a nutritionally complete elemental diet (Marsman and McBurney, 1995) and water. The elemental diet was given to minimize the effect of residual dietary fibre fermentation and SCFA production prior to surgery.

On day 5, animals were weighed and anesthetized with halothane (0.8-1.5%) following a 12 hour fast. Their abdomen, necks and midsubscalpular regions were shaved and prepared with iodine. Rats underwent superior vena cava cannulation and a swivel was placed, as outlined by Popp and Brennan (1981). In addition, all animals received an 80% small bowel resection, leaving 1-cm of jejunum distal to the ligament of Treitz and 15-cm of ileum proximal to the ileocecal junction. Bowel continuity was restored by an end-to-end jejunoileal anastomosis of interrupted 6-0 silk suture. To test for the effect of surgery on immune parameters, sham surgery animals (SHAM, n=7) underwent intestinal transection 1-cm distal to the ligament of Treitz in a similar manner as described for the resected animals.

Experimental Design

Postoperatively, animals were randomly assigned to receive either standard TPN (TPN) or TPN supplemented with SCFAs (SCFA; Table 2.1). Within these two infusion groups, rats were further randomized to receive the daily prepared sterile nutrient solutions for either 3 (TPN-3; SCFA-3) or 7 (TPN-7; SCFA-7) days. SCFAs (propionate, butyrate, and acetate) were added as sodium salts in the molar proportions found physiologically in the colon (Cummings, 1984) and at concentrations demonstrated by Koruda and associates (1988) to reduce TPN mucosal atrophy following massive small bowel resection. Diets were infused continuously (52.8 mL/day) using a Harvard infusion pump to provide 242 kJ and 425 mg nitrogen each day. Water was provided for *ad libitum* consumption throughout the study. Five rats in the TPN group and 3 rats in the SCFA group died within 6 days of surgery due to postoperative complications. Therefore, a total of 51 rats were used in the experiment.

Sham surgery animals were randomly assigned to oral elemental diets with identical nutrient composition to the standard TPN formulation for 3 or 7 days and infused with saline at the same rate that the experimental animals were receiving the nutrient infusions. Shams were otherwise exposed to the same conditions as the experimental animals. On either day 3 or 7 postsurgery, rats were weighed and anesthetized with 3% halothane. Blood was collected by cardiac puncture and rats were killed by cervical dislocation. The spleen was removed under sterile conditions and weighed.

Hematological Analysis

Complete blood counts, using a Coulter STKS instrument (Coulter Electronics, Inc., Hialeah, FA), and manual differential were performed by the staff of the Hematology Laboratory at the University of Alberta Hospital.

Preparation of Lymphocytes

Splenocytes were isolated under sterile conditions as previously described (Field et al., 1990) in Krebs-Ringer HEPES (KRH) buffer with bovine serum albumin (5 g/L). Cells were prepared and cultured in RPMI supplemented with fetal calf serum (50 g/L), 2-mercaptoethanol (2.5 µmol/L), glutamine (4000 µmol/L), penicillin (100 units/mL), streptomycin (100 µg/mL) and HEPES (25 mmol/L). Cell viability was assessed using trypan blue exclusion and was not less than 99% for all groups.

Mitogenic Responses of Immune Cells

Splenocytes (1.25×10^9/L) in the media described above were cultured in 96 well microtiter plates without mitogen or with either Concanavalin A (Con A; 5 mg/L) or Phorbol Myristate Acetate (PMA; 40 µg/L) plus Ionomycin (Iono; 0.5 µmol/L) for 38, 62, 96 and 120 hours. Cells were incubated in humidified 5% CO_2 atmosphere at 37 °C. Eighteen hours before harvesting the cells, each well was pulsed with [^{3}H]-thymidine (18.5 kBq). Cells were harvested on glass fibre filters using a multiwell harvester (Skatron, Lier, Norway) and counted using Ecolite® in a Beckman betacounter (LS 5801®, Beckman Instruments Inc., Missisauga, ON, Canada). All assays were performed in quadruplicate and stimulation indices (SI) were calculated as follows: SI= ([^{3}H]-thymidine (kBq/min) incorporated by stimulated cells - [^{3}H]-thymidine (kBq/min) incorporated by unstimulated cells)/ [^{3}H]-thymidine incorporated by unstimulated cells.

Mononuclear Cell Phenotyping

Lymphocyte subsets from spleen were characterized by immunofluorescence assay using supernatants from hybridomas secreting mouse monoclonal antibodies specific for the different rat mononuclear cell subsets. OX19 (CD5) recognizes a glycoprotein on the surface of thymocytes, T-lymphocytes and macrophages, W3/25 (CD4) recognizes rat T helper lymphocytes and macrophages, OX8 (CD8) recognizes T-cytotoxic/suppressor lymphocytes and natural killer (NK) cells, OX42 reacts with a receptor found on most monocytes and macrophages, OX12 recognizes a determinant on the rat kappa chain of immunoglobulin (Ig) on B lymphocytes and 3.2.3 reacts with rodent NK cells. Aliquots of 5×10^5 cells from individual rats were incubated for 20 minutes at 4 °C with each antibody, washed three times in 200 µL of phosphate buffered saline containing fetal calf serum (40 g/L), and incubated for another 20 minutes at 4 °C in 50 µL of a 1:300 dilution of fluoroscein isothiocyanate. Cells were washed three times and fixed in phosphate buffered saline containing paraformaldehyde (10 g/L) and analyzed on a FACScan® (Becton-Dickinson, Sunnyvale, CA) according to the relative fluorescence intensity. Resulting percentages were corrected for background fluorescence (15-20%) determined by incubating the cells with isothiocyanate-conjugated goat anti-mouse IgG only (Field, 1995).

NK Cell Cytotoxicity

NK cell cytotoxicity was determined on splenocytes isolated from each rat using a 4 hour chromium release assay (Field, 1995). NK sensitive YAC-1 cells were incubated with sodium chromate (^{51}Cr, 5.55 MBq) and seeded into 96 well v-bottom microtiter plates. Splenocytes were added in triplicate to the wells to achieve effector:target ratios between 2:1 and 100:1. Following a 4 hour incubation at 37 °C, an aliquot of the supernatant was counted in a gamma counter (Beckman Gamma 8000®, Beckman Instruments, Inc. Mississauga, ON, Canada) to determine the extent of target cell lysis. Spontaneous release was determined from target cells incubated in the absence of effector cells. Maximum release was determined from detergent lysis of labelled target cells. Cytotoxicity was calculated as follows: % specific lysis = 100 x (experimental release-spontaneous release) / (maximum release-spontaneous release). Results were also calculated using the number of NK cells present in the assay, as determined by the binding of the 3.2.3 monoclonal antibody and expressed as lytic units (LU) where one LU is the number of effector cells ($x10^{-3}$) required to cause 20% lysis of target cells.

Statistical Analysis

Data is given as means ± SEM. The effects of treatment and time on body weight and spleen weight, cell number, ^{3}[H]-thymidine incorporation, phenotyping and lytic units were analyzed using a 2-way ANOVA and a 95% confidence interval. When significant interactions ($p<0.05$) between time and diet were found, significant differences ($p<0.05$) were identified by least squares means (Steele and Torrie, 1980). The effect of time on mitogen responses was determined for each group by repeated measures ANOVA and differences determined by the Duncan multiple-range test (Steele and Torrie, 1980). NK cell activity was analyzed using a split-plot two-way ANOVA. Sham experiments were conducted after the TPN study and were not part of the original hypothesis. As there is little day to day variation in our assays we used the reponses of this group as a reference to assess the effect of surgery on immune parameters. T-tests were performed to determine if SHAM were significantly different from mean of the TPN groups. All statistical analyses were conducted using the SAS statistical package (Version 6.02, SAS Institute, Cary, NC).

RESULTS

Body and Organ Weights

All resection rats received continuous nutrient infusion throughout the study period. Average weight gain was 2.4 g/d. Weight gains did not differ among experimental groups (Table 4.1) and were similar to SHAM. Spleens were significantly ($p<0.001$) heavier on day 7 versus day 3 (Table 4.1) and were 2-fold larger than SHAM. After 7 days, spleens from the TPN-fed rats weighed more ($p<0.05$) than those from any other group. Despite weight differences, the number of cells isolated per gram of spleen was not different among groups at any time (569 ± 138, n=51) and was similar to sham-treated rats (345 ± 69, n=7). Liver weights of SCFA rats were significantly lower than TPN rats (Table 4.1). However, liver weight on a body weight basis (45 ± 20 mg/g) was not significantly different between groups (Table 4.1). Total ileal weight (mg/cm) was significantly higher in the SCFA groups when compared to the TPN groups at 3 and 7 days postsurgery (Table 4.1).

Hematological Analysis

The TPN-7 group had significantly higher total WBC counts than either SCFA group and the TPN-3 group (Figure 4.1). This increase was due to significantly ($p<0.02$) more neutrophils and monocytes (other) in the TPN-7 group. There were no significant differences in hematological parameters measured between the SCFA-3 and SCFA-7 groups.

Mitogenic Responses of Immune Cells

There were no significant difference in [^{3}H]-thymidine incorporation in response to either Con A or PMA+ Iono with diet or time (Table 4.2). The highest rate of [^{3}H]-thymidine incorporation by Con A stimulated cells was achieved by 120 hours for all groups (Table 4.2). However, the stimulation index to Con A from TPN-3 cells did not differ significantly over the 120 hours measured (Table 4.3). Splenocytes from all experimental groups did not respond well to PMA+Iono (Tables 4.2 and 4.3). Compared to cells from SHAM, the stimulation index to PMA+Iono was significantly lower for experimental rats at 38 and 62 hours (Table 4.3).

Mononuclear Cell Phenotyping

Rats in the TPN-3 group had a higher relative percent of T cells (OX 19$^+$ cells) in spleen, as compared to all other groups (Table 4.4). However, there were no differences in the relative percent of CD4$^+$ or CD8$^+$ cells or the CD4/CD8 ratio. The relative percent of B cells in the spleen did not differ among resection groups. Spleens from SCFA-3 rats contained a lower

($p<0.05$) percentage of macrophages than the other groups (Table 4.4). After 7 days, the relative percent of macrophages (OX 42^+ cells) was higher in both experimental groups (Table 4.4). The relative percent of NK ($3.2.3^+$) cells was higher for day 7 compared to day 3 and on both days higher for spleens from TPN, compared to the SCFA groups (Table 4.4). Compared to previous reports from our lab using non-surgically treated rats (Shewchuk et al., 1996), SHAM and experimental rats had a lower relative percent of T cells, both $CD4^+$ and $CD8^+$. The relative percent of B cells in spleens of SHAM rats was higher than all experimental groups. The relative percent of macrophages was approximately 2-fold greater after 7 days of TPN treatment than in SHAM rats.

NK Cell Cytotoxicity

Although the percent specific lysis was similar (Figure 4.2), 3 days postsurgery, SCFA-3 splenocytes had increased NK cell cytotoxic activity per cell when compared to the TPN group as indicated by lower LU, reflecting the need for fewer SCFA cells to induce 20% lysis of YAC-1 cells (Figure 4.3). The percent specific lysis was lower ($p<0.05$) in resected groups, compared to SHAM, at the three lowest effector:target ratios (Figure 4.2). Cytotoxic activity at ratios above 13:1 did not differ between groups.

DISCUSSION

Total parenteral nutrition is associated with intestinal atrophy and immunosuppression. SCFA are an important energy source for the enterocyte (Marsman and McBurney, 1995) and appear to stimulate gut epithelial regeneration (Yang et al., 1970; Koruda et al., 1990; Kripke et al., 1989) and could improve immunocompetence by maintaining the mucosal barrier. Immune function is sensitive to changes in nutritional status (Mainous and Deitch, 1994). Alterations in immune function observed in intravenously-fed rats were unlikely due to malnutrition. SHAM animals had smaller weight gains than the experimental groups, due to a failure to consume adequate amount of the elemental diet for 2 to 3 days following surgery. Seven days postoperatively, all parenterally-fed animals had larger spleens than

SHAM. The TPN-7 group had significantly larger spleens which contained more immune cells than any other experimental groups (Table 4.1). The reason for the splenomegaly observed in this study is unknown. Johnson and associates (1975) did not observe any changes in spleen weight in rats after three week intravenous feeding. This does not rule out the possibility that spleens were enlarged earlier, at time points consistent with those in the present study. Splenomegaly (64% increase) has been observed during interleukin-2 immunotherapy and was attributed to immune activation (Pozniak et al., 1995). Alternatively, the change in the number of splenocytes could reflect a redistribution or sequestering of lymphocytes from peripheral circulation (Olszewski and Grzelak, 1989). Liver disease is associated with an enlarged and functionally inefficient spleen (Muller and Toghill, 1992). Organ and associates (1988) studied the alterations that occur in different lymphocyte compartments after burn injury in rats and reported an initial postoperative rise (24 hour) in spleen weight with decreases in cell numbers up to 6 days after injury. Contrary to the present study, however, spleen weight and cell numbers were comparable to controls after day 6. The inclusion of SCFAs in TPN did not eliminate TPN-associated splenomegaly; however it did result in less of an increase in spleen size after 7 days.

Hematology results indicated substantial increases in all circulating immune cells in the TPN-7 rats (Figure 4.1). The functional status of these cells was not measured in the present study. The increase in total white blood cells (WBC) counts was due to increases in neutrophils and monocytes. This WBC profile may be suggestive of an inflammatory response. An inflammatory response is frequently reported during long term (>7 days) TPN when circulating pathogens translocate from the gastrointestinal tract (Alexander et al., 1991; Alverdy et al., 1988; Geland et al., 1991; Jones et al., 1991; Shou et al., 1994; Mainous et al., 1991). In the present study, the presence of bacteria in blood was not measured, however, sepsis is unlikely as rats in both groups gained weight. A larger ileal weight was observed in SCFA-supplemented rats and may be associated with a lower incidence of bacterial translocation.

Mitogen-induced lymphocyte transformations provide a useful model for the study of metabolic events associated with antigen stimulation of lymphocytes, a component of the specific immune system. Suppressed *in vitro* mitogen responses have been used as indicators of poor prognosis for a patient (Wolfe et al., 1982). Stimulation of the gut by concurrent feeding (rodent chow) in addition to TPN improved the mitogenic response of rats (Shou et al., 1994), suggesting nutrient stimulation of the gastrointestinal tract may be necessary to maintain optimal immune function. However, providing SCFAs with TPN in the present study did not improve the mitogenic responses of splenocytes to either Con A or PMA+ Iono. The suppressed mitogenic response, especially to PMA+Iono that occurred with all TPN treatment groups in comparison to SHAM animals (36 and 62 hours) parallels the observations of others (Mainous et al., 1991; Shou et al., 1994). Deitch and associates (1991) reported suppression of mitogenic response of splenocytes to Con A at 72 hours which was associated with bacterial translocation from the gut. Similarly, the stimulation index (an estimation of the response above the nonstimulated response) of Con A-stimulated lymphocytes was decreased at 24 hours after 7 days of TPN administered to patients preoperatively (Monson et al., 1988), indicating suppressed responses even in the absence of surgery. Intralipid has been reported to inhibit mitogen responses (Monson et al., 1988; Sedman et al., 1990). It is interesting that the response to Con A, a T cell mitogen, did not differ between the TPN and SCFA groups despite significantly greater (33%) percent of CD5$^+$ (T cells) in the TPN-3 group. This suggests that the environment *in vivo*, not the cell types present, affected the T-mediated immune response.

Compared to data from splenocytes of non surgically treated rats fed a semi-purified elemental diet (Shewchuk et al., 1996), all groups, including the SHAM animals, had approximately 50% lower relative percent of CD4$^+$ and a lower CD4/CD8 (T-helper cell/T-suppressor cell) ratio, suggesting this effect occurred as a result of surgery (Table 4.4). It appears that there is a population(s) of cells present in the spleen, such as immature or activated cells, that are not identified by the monoclonal antibodies used. Human studies reveal similar trends whereby immune cell numbers decrease after major trauma (Braquet, 1987; McIrvine et al., 1982). This reduction is usually characterized by decreases in the

CD4/CD8 ratio (Antonacci et al., 1984). In the present study, the CD4/CD8 ratio of experimental rats is about 60% of the same strain of rats fed a semi-purified diet (Shewchuk et al., 1996). There were no significant effects on the relative percentage of B cells with either TPN treatment which is consistent with another report that trauma does not affect B-cell numbers (Ertel and Faist, 1989). The concentration of macrophages in the experimental groups at day 7 was 10-fold higher than normal values (Shewchuk et al., 1996) and twice those of the SHAM animals. The increased concentration of macrophages observed both in the spleen and periphery is further suggestive of immune stimulation (perhaps bacterial translocation) in TPN-fed rats. SCFA had little effect on phenotypes and the distribution of immune cells in spleen.

Although there were relatively fewer NK cells in the spleen of the SCFA groups, cells from these rats demonstrated significantly higher NK cytotoxic activity on a per cell basis than either TPN groups. Although the *in vivo* cytotoxicity of NK cells was not measured, activity per cell against YAC-1 measured in splenocytes has been shown to directly correlate with *in vivo* cytotoxicity (Hanna, 1985). Lytic units for SCFA-3 were similar to SHAM rats (Figure 4.3). Long chain fatty acids, the major component of Intralipid (Gottschlich, 1992), are reported to depress NK activity (Monson et al., 1988). Pollock and associates (1991) reported an impaired NK activity in humans at 1, 3, 5 and 7 days postsurgery which was attributed to a surgery effect. Other reports have attributed the splenic NK suppression to an impairment in NK recycling capacity (Pollock et al., 1989) and to erythroblast competition with NK cells for the target binding sites in a tumor surgery model (Pollock et al., 1988). Improved cellular cytotoxic activity of NK cells in this study is likely due to the addition of SCFAs in the TPN solution as all animals were exposed to surgery and identical postsurgery conditions.

The combination of SCFAs utilized in the present study approximates those found physiologically in the colon (Cummings, 1984). Effects of individual SCFAs on immune function following surgery are not known. Ishizaka and associates (1993) examined the effect of infused acetate on immune function in patients with advanced cancer. Using similar methodology to that in the current study, they observed increased NK cytotoxicity in patients that had low initial values (Ishizaka et al., 1993). From these initial results, it is clear there is much to be elucidated regarding the role of SCFAs in the modulation of immune function. Several parameters of the nonspecific immune system were enhanced in cells from rats provided SCFA-supplemented TPN. As the nonspecific immune system is involved in primary defense capabilities against pathogens (Abbas et al., 1994) and has been reported to be decreased with TPN (Monson et al., 1988; Gogos et al., 1990; Shou et al., 1994), the addition of SCFAs to TPN may lead to quicker recoveries in clinical situations. Future studies should be designed to examine the effect of SCFAs on other components of the nonspecific immune system such as neutrophils and complement. SCFAs do not appear to have substantial effects on the depressed cell mediated immune response associated with surgery and TPN feeding. The nutritional importance of SCFAs for gastrointestinal tract is well documented (Kudsk et al., 1992; Yang et al., 1970; Koruda et al., 1990; Kripke et al., 1989), therefore, it may be reasonable to postulate that SCFAs may moderate their immune effect via maintenance of the mucosal barrier and its related immune function.

Table 4.1

Effect of SCFA-Supplemented TPN on Rat Body and Organ Weights[1]

	Initial body weight (g)	Final body weight (g)	Final ileal weight (mg/cm)	Final liver weight (g)	Final spleen weight (mg)
TPN-3	243 ± 7	300 ± 9	6.4 ± 0.6	12.2 ± 0.4	941 ± 71^{a}
SCFA-3	237 ± 8	296 ± 6	7.7 ± 0.6	11.8 ± 0.3	1033 ± 76^{a}
TPN-7	242 ± 6	311 ± 7	5.7 ± 0.6	13.1 ± 0.4	1807 ± 179^{b}
SCFA-7	228 ± 6	296 ± 6	7.5 ± 0.6	11.6 ± 0.3	1333 ± 119^{c}
Diet[2]	NS	NS	$p < 0.0204$	$p < 0.0109$	$p < 0.02$
Time[2]	NS	NS	NS	NS	$p < 0.001$
Time x Diet	NS	NS	NS	NS	$p < 0.04^{3}$
Sham	228 ± 9	242 ± 7	not measured	not measured	704 ± 59^{4}

[1] Values are expressed as mean ± SEM. Abbreviations: TPN-3, total parenteral nutrition for 3 d following surgery (n=13); TPN-7, total parenteral nutrition for 7 d following surgery (n=12); SCFA-3, short chain fatty acid supplemented total parenteral nutrition for 3 d following surgery (n=13); SCFA-7, short chain fatty acid supplemented total parenteral nutrition for 7 d following surgery (n=13); Sham, pooled d3 and d7 sham surgery values (n=7) as no significant differences existed between groups.

[2] Effect of treatment as determined by two-way ANOVA. Differences were considered to be significantly different if $p < 0.05$.

[3] Indicates that a significant interaction exists between diet and time ($p<0.05$). When a significant interaction was exists, means within a column that do not share a common superscript are significantly different ($p<0.05$) as identified by the Duncan multiple range test.

[4] Indicates that tha SHAM group is significantly different from experimental rats (all resection groups combined) as determined by a t-test.

Table 4.2 Effect of SCFA-Supplemented TPN on [^{3}H]-Thymidine Incorporation Following Intestinal Resection^{1,2}

	Con A				PMA + Iono			
	38 h	**62 h**	**96 h**	**120 h**	**38 h**	**62 h**	**96 h**	**120 h**
					(dpm)			
TPN-3	274 ± 60^{a}	322 ± 106^{a}	769 ± 688^{a}	2713 ± 1412^{b}	325 ± 583	340 ± 800	425 ± 729	444 ± 138
SCFA-3	255 ± 55^{a}	157 ± 89^{a}	1484 ± 582^{ab}	3086 ± 1193^{b}	572 ± 539	254 ± 676	1399 ± 616	360 ± 116
TPN-7	304 ± 55^{a}	220 ± 89^{a}	1174 ± 628^{b}	1508 ± 1412^{b}	1474 ± 539	2024 ± 676	1510 ± 666	448 ± 138
SCFA-7	335 ± 52^{a}	356 ± 89^{a}	1178 ± 582^{a}	5184 ± 1289^{b}	993 ± 505	578 ± 675	596 ± 666	529 ± 138
Diet^{3}	NS	NS	NS	NS	NS	NS	NS	NS
Time^{3}	NS	NS	NS	NS	NS	NS	NS	NS
Sham^{4}	538 ± 98	204 ± 26	9556 ± 2698^{4}	8371 ± 228^{4}	3601±1244$^{ab\ 4}$	973 ± 203^{bc}	3690 ± 1171^{a}	273 ± 28^{c}

[1] Values are expressed as mean ± SEM. Abbreviations: TPN-3, total parenteral nutrition for 3 d following surgery (n=13); TPN-7, total parenteral nutrition for 7 d following surgery (n=12); SCFA-3, short chain fatty acid-supplemented total parenteral nutrition for 3 d following surgery (n=13); SCFA-7, short chain fatty acid-supplemented total parenteral nutrition for 7 d following surgery (n=13); Sham, pooled d3 and d7 sham surgery values (n=4) as no significant differences existed between groups. Con A=Concanavalin A; PMA + Iono=Phorbol Myristate Acetate plus Ionomycin.

[2] For each mitogen, means within a row that do not share a common superscript are significantly ($p<0.05$) different as determined by repeated measure ANOVA procedures and differences identified by least squared mean.

[3] Effect of treatment as determined by two-way ANOVA. Differences were considered to be significantly different if $p<0.05$. There was no significant interaction between Time and Diet.

[4] Indicates that the SHAM group is significantly different ($p<0.05$) from all resection groups combined as determined by a t-test.

Table 4.3

Effect of SCFA-Supplemented TPN on Lymphocyte Stimulation Index Following Intestinal Resection[1,2]

	Con A				PMA + Iono			
	38 h	**62 h**	**96 h**	**120 h**	**38 h**	**62 h**	**96 h**	**120 h**
TPN-3	0.58 ± 0.19	0.39 ± 0.14	2.66 ± 1.59	5.70 ± 4.37	0.62 ± 0.80	0.75 ± 1.24	0.60 ± 1.22	0.42 ± 0.21
SCFA-3	0.24 ± 0.18^{a}	0.12 ± 0.12^{a}	4.60 ± 1.34^{ab}	8.62 ± 3.70^{b}	0.78 ± 0.74^{a}	0.26 ± 1.05^{a}	3.03 ± 1.03^{b}	0.35 ± 0.17^{a}
TPN-7	0.31 ± 0.18^{a}	0.22 ± 0.12^{a}	3.11 ± 1.45^{a}	10.9 ± 4.37^{b}	1.90 ± 0.74	2.95 ± 1.05	2.81 ± 1.11	0.95 ± 0.21
SCFA-7	0.17 ± 0.16^{a}	0.36 ± 0.12^{ab}	4.96 ± 1.^{ab}	10.8 ± 4.37^{c}	1.53 ± 0.69	1.15 ± 1.05	1.93 ± 1.11	0.82 ± 0.21
Diet[3]	NS	NS	NS	NS	NS	NS	NS	NS
Time[3]	NS	NS	NS	NS	NS	NS	NS	NS
Sham	0.86 ± 0.09^{a}	0.42 ± 0.11^{a}	6.47 ± 3.81^{a}	27.2 ± 11.9^{b}	10.6 ± 3.0^{a}	5.90 ± 1.44^{ab}	8.43 ± 2.63^{ab}	0.47 ± 0.17^{b}

[1] Stimulation Index = (amount of [^{3}H] thymidine incorporated by stimulated cells - amount of [^{3}H] thymidine incorporated by unstimulated cells) / amount of [^{3}H] thymidine incorporated by unstimulated cells.

[2] Values are expressed as mean ± SEM. Abbreviations: TPN-3, total parenteral nutrition for 3 d following surgery (n=13); TPN-7, total parenteral nutrition for 7 d following surgery (n=12); SCFA-3, short chain fatty acid supplemented total parenteral nutrition for 3 d following surgery (n=13); SCFA-7, short chain fatty acid supplemented total parenteral nutrition for 7 d following surgery (n=13); Sham, pooled d3 and d7 sham surgery values as no significant differences existed between groups (n=4); Con A, concanavalin A; PMA + Iono, Phorbol Myristate Acetate + Ionomycin.

[3] Effect of treatment as determined by 2-way ANOVA. Differences were considered to be significantly different if $p<0.05$.

[4] For each mitogen, means with a row that do not share a common superscript are significantly ($p<0.05$) different as determined by ANOVA

Table 4.4 Effect of SCFA-Supplemented TPN on Phenotypes in Spleen Following Intestinal Resection[1]

	OX19 (CD5$^+$ cells)	W3-25 (CD4$^+$ cells)	OX8 (CD8$^+$ cells and NK cells)	OX12 (B cells)	OX42 (Macrophages)	3.2.3. (NK cells)	CD4/CD8 ratio
				(% of Total)			
TPN - 3	$40 \pm 3^{a3*}$	$14 \pm 2^*$	10 ± 2	$20 \pm 3^*$	9 ± 1^a	8 ± 1	1.1 ± 0.2
SCFA - 3	30 ± 2^b	$14 \pm 2^*$	11 ± 2	$19 \pm 3^*$	5 ± 1^b	$5 \pm 1^*$	1.5 ± 0.3
TPN - 7	31 ± 3^b	$15 \pm 2^*$	12 ± 2	$20 \pm 4^*$	$12 \pm 1^{a*}$	$10 \pm 1^*$	1.3 ± 0.3
SCFA - 7	30 ± 2^b	$15 \pm 2^*$	14 ± 1	$18 \pm 3^*$	11 ± 1^a	7 ± 1	1.4 ± 0.2
Diet[2]	$p < 0.05^3$	NS	NS	NS	NS^3	$p < 0.001^3$	NS
Time[2]	NS^3	NS	NS	NS	$p < 0.001^3$	$p < 0.001^3$	NS
Sham[4]	$28 \pm 1^*$	$20 \pm 1^*$	11 ± 1	$30 \pm 1^*$	$6 \pm 1^*$	$7 \pm 1^*$	1.2 ± 0.3

[1] Values are expressed as mean ± SEM. Abbreviations: TPN-3, total parenteral nutrition for 3 d following surgery (n=13); TPN-7, total parenteral nutrition for 7 d following surgery (n=12); SCFA-3, short chain fatty acid supplemented total parenteral nutrition for 3 following surgery (n=13); SCFA-7, short chain fatty acid supplemented total parenteral nutrition for 7 d following surgery (n=12); Sham, pooled d3 and d7 sham surgery values (n=7) as no significant differences existed between groups; Reference, value from splenocytes of rats fed a semipurified elemental diet; NK cell, natural killer cell.

[2] Effect of treatment as determined by two-way ANOVA. Differences were considered to be significantly different if $p < 0.05$. There was no significant interaction between Time and Diet.

[3] Indicates that a significant interaction exists between diet and time ($p<0.05$). When a significant interaction exists, means within a column that do not share a common superscript are statistically different ($p < 0.05$) as identified by the Duncan's multiple range test.

[4] * indicates SHAM group is significantly different ($p<0.05$) from resection group marked with * as determined by a t-test.

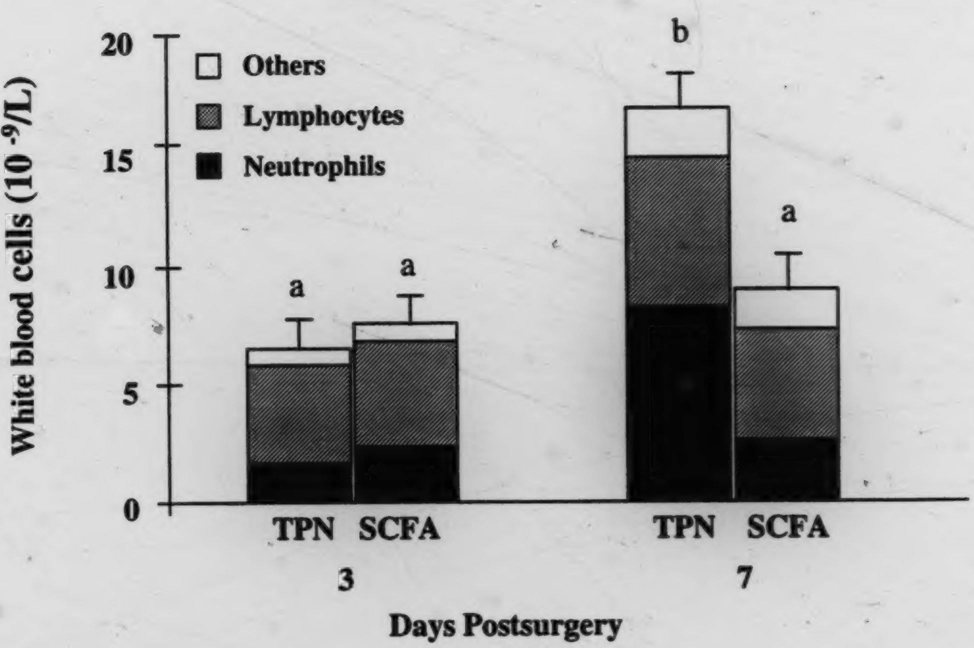

Figure 4.1 Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on White Blood Cell Concentrations 3 and 7 Days Following Intestinal Resection in Rats.

White blood cell concentration was significantly elevated ($p<0.05$) following 7 days of TPN (TPN-7;n=12) when compared to all other groups (TPN-3, n=13; SCFA-3, n=13; SCFA-7, n=13). Bars (mean ± SEM) that do not share a common superscript are significantly different as determined by a two-way ANOVA and least squares means.

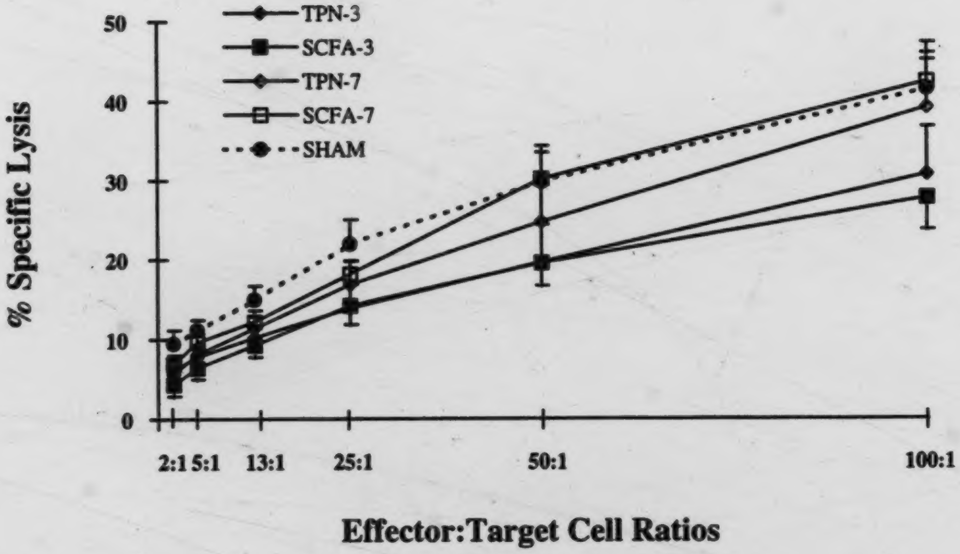

Figure 4.2 Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Splenocyte Natural Killer Cell Cytotoxic Activity 3 and 7 Days Following Intestinal Resection in Rats.

Natural killer cytotoxic activity is expressed as % specific lysis = 100 x (experimental release of ^{51}Cr from ^{51}Cr-labelled YAC-1 cells - spontaneous release)/(maximum release - spontaneous release). Points represent % specific lysis at each effector:target ratio. There was no significant difference between resected groups. At effector:target ratios 2:1, 5:1 and 13:1 the % specific lysis by resected groups was significantly ($p<0.05$) less than SHAM. Values represent mean ± SEM (n=13/experimental group except TPN-7, n=12 and n=7 in the SHAM treatment).

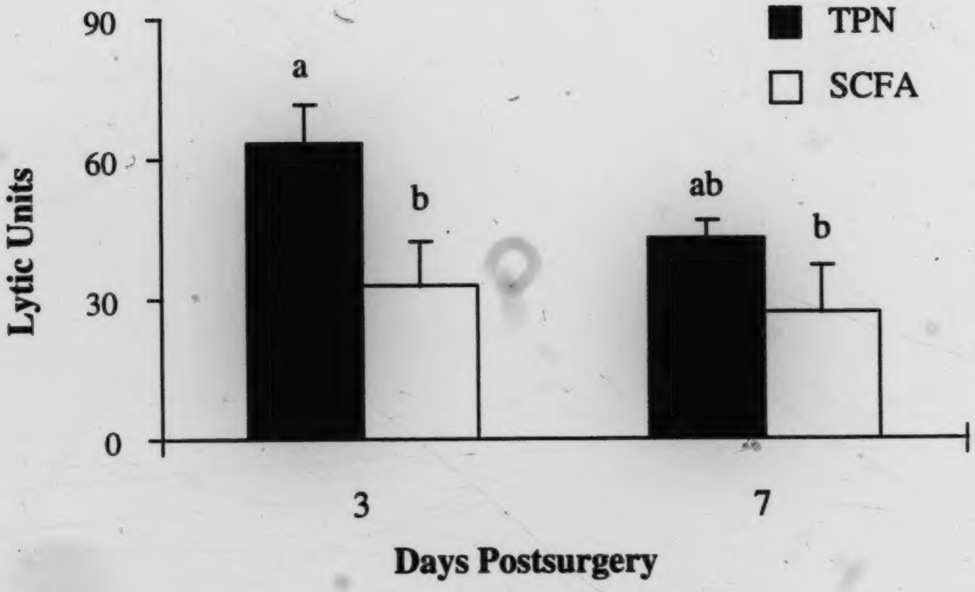

Figure 4.3 Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Rat Splenocyte Natural Killer Cell Cytotoxic Activity Expressed as Lytic Units (the number ($x10^{-3}$) of $3.2.3^{+}$ cells required to cause 20% lysis of YAC-1 cells) at 3 and 7 Days Following Intestinal Resection in Rats.

Bars represent mean ± SEM (n=13/experimental group except TPN-7, n=12 and n=7 in the SHAM treatment). SCFA-supplemented TPN enhanced NK cell cytotoxicity both 3 and 7 days following surgery as fewer ($p<0.05$) cells were required to cause 20% lysis of the target cells.

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CHAPTER 5

SKELETAL MUSCLE CATABOLISM IS DECREASED FOLLOWING INTESTINAL RESECTION BY SHORT-CHAIN FATTY ACID-SUPPLEMENTED TOTAL PARENTERAL NUTRITION [8,9]

INTRODUCTION

The metabolic response to surgical trauma is characterized by protein catabolism and negative nitrogen balance (Cuthbertson, 1931). Skeletal muscle, comprising 40% of the total protein in the body (Young, 1970), represents the largest pool of free amino acids in the human body (Bergström et al., 1974) and acts as an energy and amino acid store during conditions of trauma (Sjölin et al., 1990; Wolfe et al., 1989). Amino acids are released from muscle and transported to other tissues for oxidation, gluconeogenesis, and liver protein synthesis (Clowes et al., 1980; Askanazi et al., 1978; Ryan, 1976; Souba et al., 1985; Daniel et al., 1977; Felig and Wahren, 1974). Glutamine and alanine account for 60% of the amino acid nitrogen released by muscle during stress states (Souba et al., 1990). Despite accelerated skeletal muscle release, plasma concentrations of glutamine are diminished, indicating accelerated uptake in non-muscle tissues. The small intestine is the principal organ of glutamine uptake and metabolism in the body in both the normal and stress states (Souba, 1993).

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Following gastrointestinal surgery, total parenteral nutrition (TPN) is frequently administered to prevent diarrhea, dehydration, electrolyte disturbances, malabsorption and progressive malnutrition. Postoperative administration of TPN improves nitrogen balance but equilibrium is unlikely in the initial days following surgery (Vinnars et al., 1969; Elwyn, 1980). Even when fat gain and resynthesis of glycogen stores are observed, it is difficult to promote the resynthesis of body proteins with TPN (Warnold et al., 1988; Streat et al., 1987; Hill et al., 1991). As TPN alone cannot protect against protein catabolism and preserve lean body mass, research in several labs have strived to optimize the composition of current intravenous nutrient solutions (Hammarqvist et al., 1989, 1991; Babst et al., 1993; Wernerman et al., 1990). TPN supplemented with short chain fatty acids (SCFAs) is reported to support gastrointestinal structure and function following intestinal surgery (Chapters 2 and 3; Koruda et al., 1988), however it's impact on postsurgical skeletal muscle wasting is unknown. We hypothesized that systemic provision of SCFAs following intestinal resection would reduce the gastrointestinal tracts demand for serosally-derived glutamine as an oxidative fuel and spare skeletal muscle catabolism.

MATERIALS AND METHODS

Animals

All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care. Forty-seven adult male Sprague-Dawley rats (weighing 236 ± 4 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet and drinking water. The elemental diet was given to minimize dietary fiber fermentation and subsequent SCFA production in the gastrointestinal tract prior to the onset of the experiment.

Surgical Model

Prior to surgery, animals underwent a 12 hour food restriction, were weighed and anesthetized with halothane. Their abdomen, neck and midsubscapular region were shaved and prepared with iodine. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (1981). Subsequently, all animals were subjected to an 80% proximal jejunoileal resection as previously described (Chapter 2).

Postoperative Care and Nutrient Solutions

Postoperatively, animals were randomly assigned to receive either standard TPN or TPN supplemented with SCFAs. Within these two diet groups, animals were further randomized to receive the parenteral solutions for either 3 or 7 days. The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain optimal sterile conditions and were filter sterilized before infusion (0.22μm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemicals, St. Louis, MO) in the concentrations used by Koruda and associates (1988) and the molar proportions found physiologically in the colon of man (Cummings, 1984). The diets were infused daily to provide 205 nonprotein kJ and 0.4 g nitrogen. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Sample Collection

Urine was collected daily into containers containing 0.5 mL of 1*N* H_2SO_4, weighed and stored at -20°C until analyzed. On the third (TPN-3, n=12; SCFA-3, n=12) and seventh (TPN-7, n=11; SCFA-7, n=12) postoperative days, animals were weighed and anesthetized with halothane. Following cardiac puncture, animals were killed by exsanguination. Epitrochlearis muscles were surgically removed from each foreleg and weighed.

In Vitro Measurement of Protein Turnover

Epitrochlearis muscles were dissected intact immediately after animals were killed. Methods of protein turnover used routinely in our laboratory have been described in detail elsewhere (Tischler et al., 1982; Strelkov et al., 1989). Muscles were weighed and preincubated in individual flasks containing 3 mL of a modified Krebs Ringer Bicarbonate medium (KRB) composed (in m*M*) of 119 NaCl, 4.82 KCl, 1.25 $MgSO_4$, 1.0 $CaCl_2$, 1.24 NaH_2PO_4, 25 $NaHCO_3$, 2.0 N-2-hydroxyethylpiperazine-N'-N-2-ethane sulfonic acid (HEPES)/NaOH (pH 7.4), 5 glucose and a complete mixture of amino acids present at concentrations in normal rat plasma, except tyrosine which was present at 1 nmol/mL. One muscle from each animal was incubated in the standard media outlined above, whereas the contralateral muscle was incubated in the same media containing 0.1 U/mL bovine pancreatic insulin. After 30 minutes, muscles were transferred to fresh medium of identical composition also containing phenylalanine (1.0 m*M*) and ^{3}H-phenylalanine (0.05 µCi/mL) for a period of 3 hours. All tissues were incubated at 35°C in media equilibrated with 95% O_2/5% CO_2. After incubation, media and muscle tissue were stored at -70°C for future determination of protein synthesis and net protein degradation. Protein synthesis was estimated by quantifying the amount of phenylalanine incorporated into trichloroacetic acid precipitated proteins. Net protein degradation rate was assessed by quantifying the net tyrosine release as this amino acid is neither synthesized nor degraded by the tissue. Isolated muscle preparations are in negative nitrogen balance *in vitro*, however changes observed in this system qualitatively reflect that which occurs *in vivo*. For example, Strelkov and associates (1989) using an *in vitro* approach and Tessitore and associates (1993) using an *in vivo* approach observed a similar degree of stimulation of muscle protein degradation in rats bearing the Yoshida ascites hepatoma.

Metabolite Assays

Urinary 3-methylhistidine (3-MH) concentration, an index of muscle degradation (Harris and Milne, 1981; Nishizawa et al., 1989), was determined by HPLC using the method described by Scott and associates (1993) with some modifications. Briefly, 200 µL of internal standard (0.1 m*M* histidinol) was added to 400 µL of standard (0.1 m*M* 3-MH) or 400 µL of urine samples. The samples were deproteinized with 100 µL of 3.0 *M* $HClO_4$ and centrifuged at 3,000 x g for 15 minutes. A 500 µL portion of the supernatant was hydrolyzed by adding 500 µL of 12 *M* HCl and incubating for 2 hours at 110°C. To the hydrolysates (1.0 mL) 1.0 mL 6 *M* NaOH and 1.0 mL 2 *M* $Na_2B_4O_7$ was added. While being vortexed, the samples were derivatized with 1.0 mL of fluorescamine solution (160 mg of fluorescamine/100 mL of acetonitrile). Samples were allowed to sit for a few seconds to use up the excess fluorescamine and the 1.0 mL 2 *M* HCl was added. The samples were then incubated at 90°C for 45 minutes and extracted twice with diethyl ether (Fisher Scientific, Fair Lawn, NJ). The samples were analyzed using a Varian Model 5000 Liquid chromatograph with a Varian 2050 spectrofluorometer detector and a Varian 9090 autoanalyzer (Varian Instruments, Walnut Creek, CA). The binary gradient used in the sample analysis was as follows: solvent A was 2.5 m*M* acetylnitrimethylammonium bromide and 0.1 *M* sodium acetate buffer (pH 6.5), and solvent B was 2.5 m*M* acetylnitrimethylammonium bromide in 90% acetonitrile (pH 6.5). The gradient rose from 25 to 80% of solvent B over 12 minutes and held at that levels for 2 minutes and then back to 25% in 0.5 minutes. Total analysis time was 18 minutes per sample.

Plasma amino acids were measured using HPLC based on the method of Jones and Gilligan (1983) as modified by Sedgewick and associates (1991). Nitrogen contents of urine were analyzed by the Kjeldahl method (Bradstreet, 1965). Plasma insulin concentration was measured at the Muttart Diabetes Research Center, University of Alberta using a commercial double antibody radioimmunoassay kit (Kabi Pharmacia Dianostics AB, Uppsala, Sweden 1983).

Statistics

Differences between treatments were determined using a randomized block analysis of variance. Sources of variation were blocks (surgery day = 12), diet (d=2), time (t=2) and diet interacted with time. Comparisons within the diet by time interaction were made using student's t-test. Statistical significance was defined as $p \leq 0.05$. Computations were done using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC). All data are presented as mean ± SEM.

RESULTS

Body Weight

All animals received continuous nutrient infusion throughout the study period. The nutrient solutions were designed and administered to meet energy and protein requirements necessary for growth in rats of this size. Postsurgical changes in body weight were not different between diet groups (Day 3, +7.8 ± 2.8 g since day 0; Day 7, +18.0 ± 2.4 g since day 0).

Nitrogen Balance

Cumulative nitrogen balance did not differ between control TPN and SCFA-supplemented TPN groups at either time point following surgery (Table 5.1). Duration of infusion influenced nitrogen balance as within each diet group, the nitrogen balance was lower ($p=0.0001$) in the 7 day than the 3 day group (Table 5.1).

Skeletal Muscle Loss

Epitrochlearis muscle weights were lower ($p<0.05$) following 7 days of standard TPN (42.6±2.3 mg) than those from the 7 day SCFA supplemented TPN animals (49.8±2.3 mg). When compared to the muscle weights obtained 3 days postsurgery, muscle loss was significantly greater ($p<0.05$) in the TPN group (-7.4±3.7 mg) with atrophy occuring at approximately 4% per day. The muscle mass of the SCFA-supplemented group remained stable (+2.7±2.6 mg) between day 3 and 7 (Figure 5.1).

Urinary 3-methylhistidine excretion

Urinary 3-MH data is presented in Figure 5.2. In the first 3 days following surgery, there were no differences in urinary 3-MH excretion due to SCFA supplementation. However, by postsurgical day 7, the concentration of urinary 3-MH was significantly lower in the SCFA-supplemented TPN group when compared to all other groups.

In Vitro Protein Turnover

In the presence of insulin, net protein degradation was not influenced by SCFA supplementation or length of infusion. In the absence of insulin, the rate of net protein degradation was significantly lower ($p=0.05$) in the 7 day SCFA-supplemented group when compared to the 3 day SCFA-supplemented group, whereas net protein degradation rate did not differ between the 3 and 7 day control TPN groups (Table 5.2). Protein synthesis rate was not influenced by SCFA supplementation or length of infusion in the presence or absence of insulin (Table 5.2).

Plasma amino acid concentrations

Plasma amino acid concentrations are presented in Table 5.3. Notably, plasma glutamine was significantly higher ($p<0.02$) in the SCFA-supplemented group both 3 and 7 days following surgery. Plasma asparagine, tyrosine and methionine were also influenced by diet but were lower in the SCFA-supplemented groups. Duration of infusion resulted in higher plasma aspartate, taurine, alanine, phenylalanine, isoleucine, leucine and ornithine concentrations. Glycine was also higher in both 7 day than 3 day groups, however the 7 day SCFA-supplemented group was significantly higher than the control TPN 7 day group.

Plasma Insulin Concentration

Plasma insulin concentration was not influenced by SCFA-supplemented TPN or duration of nutrient infusion following surgery (Figure 5.3).

DISCUSSION

The supplementation of TPN with the SCFAs acetate, propionate, and butyrate following massive small bowel resection protects against skeletal muscle atrophy in the initial days following surgery. The maintenance of epitrochlearis muscle mass in the presence of systemic SCFAs was further supported by lower 3-MH excretion, reduced rates of protein degradation in the absence of insulin and increased plasma glutamine concentration.

The animals in this study exhibited a generalized catabolic response that is to be expected following major abdominal surgery (Cuthbertson, 1931). Amino acids are being supplied systemically by the parenteral nutrient solutions, however whole body amino acid metabolism is likely modified as 80% of the gut has been recently resected. Changes in plasma amino acid concentrations with time likely reflect mobilization from skeletal muscle or excess supply in the parenteral solutions. Increased plasma concentrations of branched-chain and aromatic amino acids are a hallmark of post-surgical catabolism (Vinnars et al., 1975). By contrast, increased plasma levels of glycine and serine may be a consequence of excess supply in the parenteral formulation due to unusual utilization induced by massive small bowel resection.

The higher plasma glutamine in the SCFA groups implies a sparing effect of SCFAs on glutamine requirements following intestinal injury. Glutamine and SCFAs have both been identified as important oxidative fuels for the gastrointestinal tract (Windmueller and Spaeth, 1978; Mallet et al., 1986; Ardawi and Newsholme, 1985; Sakata, 1987). SCFAs are derived from large bowel fermentation, where SCFA production increases with the intake of dietary fiber (McBurney et al., 1988). However, during the provision of TPN, fermentable substrate will not reach the large bowel to stimulate microbial fermentation and SCFA production. Theoretically, the gastrointestinal tract will become deprived of luminally-derived SCFAs as an oxidative fuel and become more dependent on serosally-supplied glutamine. Following laparotomy, the glutamine uptake by the gastrointestinal tract increases more than 50% (Souba et al., 1990). As glutamine and alanine account for 60% of the amino acids released by skeletal muscle to the splanchnic bed (Souba and Wilmore, 1983), the provision of SCFAs as an alternative gastrointestinal fuel may reduce skeletal muscle proteolysis by modulating the oxidation of glutamine for energy. Investigations examining communication between the gastrointestinal tract and skeletal muscle may help elucidate mediators in interorgan nutrient partitioning.

Although controversial (Tjäder et al., 1996), the loss of muscle mass following abdominal surgery is generally thought to be due to a decrease in the rate of protein synthesis (Stjernström et al., 1981, 1986; Wernerman et al., 1986) since degradation is unaffected (Rennie et al., 1984; Vinnars et al., 1980; McNurlan et al., 1991). We suspect that in the current model, systemic SCFAs alter protein degradation, as opposed to synthesis rate. In the absence of insulin, protein degradation rate declined between day 3 and 7 following surgery in the SCFA-supplemented group. Furthermore, urinary 3-MH excretion, an *in vivo* marker of skeletal muscle catabolism was significantly reduced in the SCFA-supplemented TPN group over the 7 days following surgery. Indeed, if skeletal muscle is degraded to supply the gastrointestinal tract with glutamine for energy purposes, the replacement of glutamine with an alternative gastrointestinal fuel may eliminate the motive for degradation.

Total body nitrogen balance was unaffected by SCFA-supplemented TPN indicating that skeletal muscle was spared at the expense of other, as yet identified sources of amino acids. This observation confirms that of Koruda and associates (1988) using a similar massive small bowel resection model. The apparent discrepancy may be attributable to improved nitrogen economy in tissues other than skeletal muscle within the control TPN groups.

In the normal situation, SCFA fermentation in the colon may provide a constant, continual oxidative substrate that does not fluctuate in the same diurnal pattern as nutrients absorbed from a meal. The continuous infusion of SCFAs in the current study may mimick this normal state and reduce gut-derived signals indicating substrate deprivation thus sparing serosally-derived fuels. Other methods of TPN administration which are used to approximate normal eating habits influence protein metabolism. For example, sequentially administered TPN results in less efficient nitrogen utilization when fat and amino acids are given without simultaneous glucose, as compared with a continuous administration of all TPN components (Hyltander et al., 1993; Sandstrom et al., 1995). By comparison, when TPN is given in a pulsative mode for 30 minutes six times daily, nitrogen retention is superior (Hyltander et al., 1993). The addition of SCFAs to continuous nutrient infusions may normalize endogenous "biological factors" associated with the physiologic and metabolic response to luminal nutrition.

The mechanism by which systemic SCFAs protect against skeletal muscle catabolism are not directly addressed in the current study, but may include: 1) a direct effect of SCFAs on skeletal muscle protein turnover; 2) an increased level of protein synthesis resulting from higher levels of plasma glutamine concentration (MacLennan et al., 1987); or 3) an indirect effect of SCFAs that may be mediated by distant organs via interorgan signaling molecules. Using this model, we have previously reported that SCFA-supplemented TPN prevents an abnormal increase in white blood cell concentration and enhances NK cell cytotoxicity (Chapter 4). Sepsis and the liberation of sepsis-related mediators such as endotoxin, IL-1 and TNF-α have been shown to mediate catabolism (Austgen et al., 1992; Millward et al., 1989). Future studies will determine if SCFAs directly influence the skeletal muscle and immune systems or if these effects are mediated by the gastrointestinal tract and interorgan signaling.

This study confirms that the beneficial effects of SCFA-supplemented TPN are not limited to the gastrointestinal tract following surgery trauma. SCFA-supplemented TPN counteracts skeletal muscle catabolism and is associated with reduced protein degradation rates, urinary 3-MH excretion and sparing of plasma glutamine. Future investigations should examine interorgan signaling and substrate preferences aimed at reducing postsurgical protein catabolism through optimized nutritional support.

Table 5.1

Effect of SCFA-Supplemented TPN on Nitrogen Balance 3or 7 Days Following Intestinal Resection[1]

	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=11)	**Day 3** (n=12)	**Day 7** (n=12)	DIET	TIME
Nitrogen Intake	1.7	1.7	1.7	1.7	NS	NS
Urinary Nitrogen	1.0 ± 0.5	3.6 ± 0.5	0.8 ± 0.5	2.7 ± 0.5	NS	$p = 0.0001$
Nitrogen Balance	0.7 ± 0.5	-2.0 ± 0.5	0.9 ± 0.5	-0.9 ± 0.5	NS	$p = 0.0001$

[1] Data expressed in grams/kg body weight/day (means±SEM).

Table 5.2

Effect of SCFA-Supplemented TPN on Protein Synthesis and Degradation 3 or 7 Days Following Intestinal Resection[1,2]

	TPN		TPN+SCFA			
	Day 3 (n=12)	**Day 7** (n=11)	**Day 3** (n=12)	**Day 7** (n=12)	DIET	TIME
Without Insulin						
tyrosine release (pmol tyr/mg muscle/3 hr)	408 ± 41^{ab}	362 ± 41^{b}	469 ± 36^{a}	352 ± 36^{b}	NS[2]	NS[2]
phenylalanine incorporation (pmol phe/mg muscle/3 hr)	102 ± 14	112 ± 13	116 ± 13	100 ± 12	NS	NS
With Insulin						
tyrosine release (pmol tyr/mg muscle/3 hr)	358 ± 45	387 ± 45	384 ± 41	329 ± 40	NS	NS
phenylalanine incorporation (pmol phe/mg muscle/3 hr)	135 ± 13	121 ± 13	112 ± 13	109 ± 12	NS	NS

[1] Values are means ± SEM.

[2] Indicates a significant interaction between diet and time. When a significant interaction exists, means within a row with different superscripts are significantly different ($p<0.05$).

Table 5.3 Effect of SCFA-Supplemented TPN on Plasma Amino Acid Concentrations Following Intestinal Resection[1,2]

	TPN		TPN+SCFA			
	Day 3 (n=11)	Day 7 (n=12)	Day 3 (n=11)	Day 7 (n=12)	DIET	TIME
Aspartic acid	10.3 ± 1.4	14.7 ± 1.3	12.9 ± 1.2	16.8 ± 1.4	p < 0.08	p < 0.003
Glutamine	630 ± 41	604 ± 40	672 ± 39	764 ± 43	p < 0.02	NS
Asparagine	42.8 ± 2.2	41.1 ± 2.1	35.8 ± 2.0	39.3 ± 2.3	p < 0.05	NS
Histidine	68 ± 27	77 ± 26	114 ± 24	73 ± 28	NS	NS
Glycine	469 ± 33^{a}	602 ± 32^{b}	427 ± 30^{a}	714 ± 34^{c}	NS[2]	p = 0.0001[2]
Arginine	113 ± 9	123 ± 9	108 ± 8	104 ± 10	NS	NS
Taurine	62 ± 6	87 ± 6	50 ± 5	83 ± 6	NS	P = 0.0001
Alanine	345 ± 39	394 ± 38	290 ± 36	424 ± 41	NS	p < 0.02
Tyrosine	55 ± 3	50 ± 3	47 ± 3	46 ± 3	p = 0.05	NS
Tryptophan	36 ± 2	40 ± 2	36 ± 2	36 ± 2	NS	NS
Methionine	58 ± 3	59 ± 3	52 ± 3	50 ± 3	p < 0.001	NS
Valine	109 ± 11	126 ± 11	103 ± 10	125 ± 11	NS	P = 0.07
Phenylalanine	65 ± 3	78 ± 3	66 ± 3	68 ± 3	NS	p = 0.03
Isoleucine	58 ± 5	72 ± 5	50 ± 4	66 ± 5	NS	p = 0.001
Leucine	69 ± 8	82 ± 8	63 ± 7	79 ± 8	NS	p = 0.06
Ornithine	49 ± 5	64 ± 5	44 ± 5	57 ± 5	NS	p < 0.01
Serine	308 ± 14	397 ± 13	235 ± 12	351 ± 14	p = 0.0001	p = 0.0001
Threonine	168 ± 14	180 ± 14	135 ± 12	111 ± 14	p = 0.0003	NS
Citrulline	20 ± 2	19 ± 2	22 ± 2	18 ± 2	NS	NS
Lysine	290 ± 33	221 ± 33	302 ± 30	185 ± 34	NS	p < 0.01

[1] Expressed in nmol/mL (mean ± SEM).

[2] Indicates a significant interaction between diet and time. When a significant interaction exists, means within a row with different superscripts are significantly different ($p<0.05$).

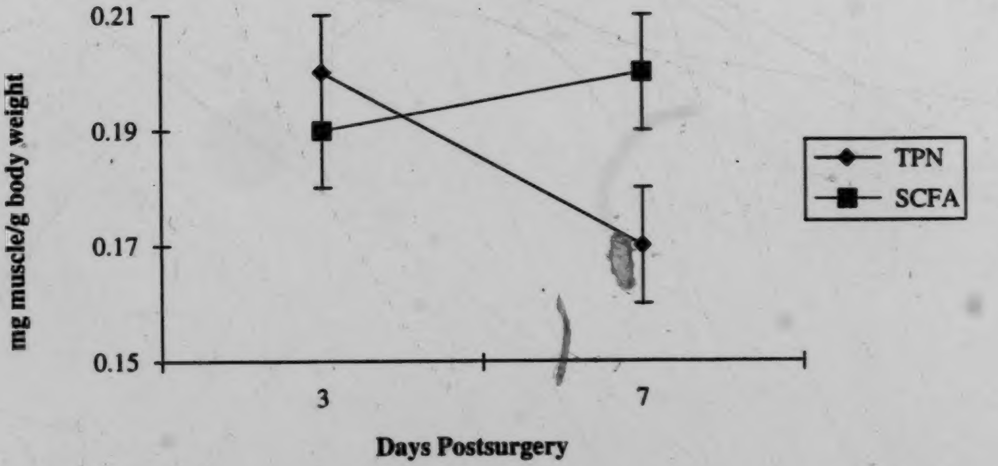

Figure 5.1 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Epitrochlearis Muscle Weight 3 and 7 Days Following Intestinal Resection.

Values are means ± SEM. Epitrochlearis muscle weight declined ($p<0.05$) between postsurgical day 3 and 7 in the control TPN group (TPN-3, n=12; TPN-7, n=11). The epitrochlearis muscle of the SCFA-supplemented TPN groups remained weight stable between the 3 and 7 day groups (SCFA-3, n=12; SCFA-7, n=12).

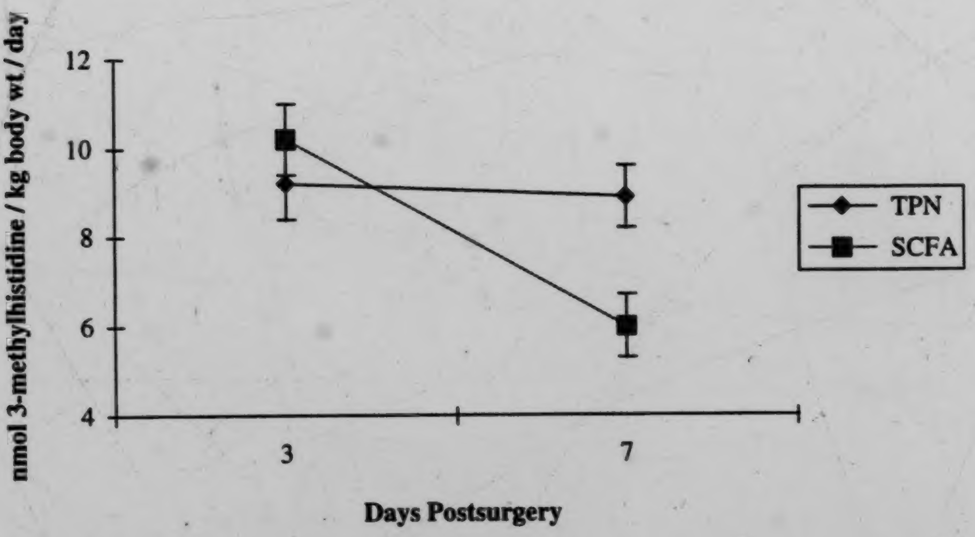

Figure 5.2 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Urinary 3-Methylhistidine Excretion 3 and 7 Days Following Intestinal Resection.

Values are means ± SEM. Urinary 3-methylhistidine excretion decreased ($p<0.05$) between postsurgical day 3 and 7 in the SCFA supplemented TPN group (SCFA-3, n=12; SCFA-7, n=12). The urinary 3-methylhistidine excretion in the control TPN groups did not differ between the 3 and 7 day groups (TPN-3, n=12; TPN-7, n=11).

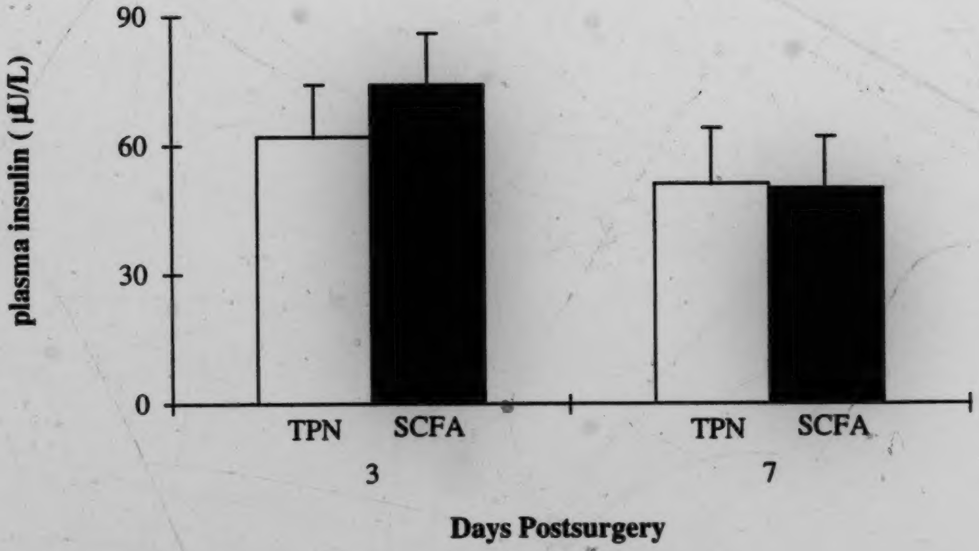

Figure 5.3 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Plasma Insulin Concentration 3 and 7 Days Following Intestinal Resection.

Values are means ± SEM. Plasma insulin concentration was not influenced by SCFA-supplemented TPN or duration of infusion following surgery (TPN-3, n=12; SCFA-3, n=11; TPN-7, n=12; SCFA-7, n=12).

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CHAPTER 6

NATURAL KILLER CELL CYTOTOXICITY AND SKELETAL MUSCLE TURNOVER ARE NOT DIRECTLY AFFECTED BY SYSTEMIC SHORT-CHAIN FATTY ACIDS: A POSSIBLE ROLE FOR INTERLEUKIN-1β? [10]

INTRODUCTION

Following massive small bowel resection, the remnant intestine undergoes adaptive morphological (i.e. dilation, lengthening and thickening) and functional (i.e. hyperproliferative, digestive and absorptive) changes (Wilmore et al., 1971; Sigalet et al., 1992). Prior to these adaptive events, patients with "short bowel syndrome" present with diarrhea, weight loss, electrolyte abnormalities and multiple nutritional deficiencies (Dudrick et al., 1991). Following surgery, accelerated skeletal muscle proteolysis and translocation of amino acids to visceral organs occurs (Souba and Wilmore, 1983). In addition, surgery itself has an immunosuppressive effect by decreasing helper and suppressor lymphocyte populations (Hansbrough et al., 1984), granulocyte function (Christon, 1985) natural killer (NK) cell cytotoxicity (Pollock et al., 1991) and delaying hypersensitivity skin-test response (Johnson et al., 1987).

The use of total parenteral nutrition (TPN) has afforded indefinite survival and thus enhanced the prognosis of patients with short bowel syndrome. However, the successful role of TPN in providing nutritional support for these patients somewhat obscures the reduction in intestinal adaptation and mucosal atrophy that occurs if the intestine is robbed of the stimulus of continued enteral nutrition (Booth, 1994; Johnson et al., 1975; Wilmore et al., 1988). The administration of luminal nutrients seems necessary to stimulate morphological (Johnson et al., 1975; Levine et al., 1974; Hosada et al., 1989; Rossi et al., 1993; Inoue et al., 1993; Miura et al., 1992) and functional (Levine et al., 1974; Inoue et al., 1993; Miura et al., 1992) aspects of intestinal adaptation. TPN is also associated with immunosuppression as reflected by an increased incidence of infections in TPN- versus enerally-fed patients (Kudsk et al., 1992; Mainous et al., 1991; McGeer et al., 1990; Moore et al., 1992). The mechanism for increased susceptibility to infection and immunosuppression associated with TPN is not known, however detrimental changes have been reported in both innate (Cleary and Pickering, 1983; Fraser et al., 1983; Hawley and Gordon, 1976; Monson et al., 1988; Pollock et al., 1991) and cell-mediated immunity (Gogos et al., 1990; Kinsella et al., 1990; Sedman et al., 1991; Shou et al., 1994; Wolfe et al., 1982). The inability of the immune system to resist infection can lead to hypermetabolism and sepsis. These multiorgan abnormalities have prompted the addition of specific nutrients to current parenteral nutrient formulations in an attempt to enhance outcomes in the critically ill.

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[10] A version of this chapter has been submitted as a "Preliminary Report". Tappenden KA, Baracos VE, Field CJ, McBurney MI. JPEN, December, 1996.

Short chain fatty acids (SCFAs) are a group of preferred gastrointestinal fuels that are readily absorbed by intestinal mucosa, are relatively high in energy, are readily metabolized by intestinal epithelium and liver, stimulate sodium and water absorption in the colon, and are trophic to the intestinal mucosa (Rombeau and Kripke, 1990). The addition of SCFAs to TPN has been shown to prevent TPN-associated mucosal atrophy (Koruda et al., 1990) and enhanced structural and functional markers of adaptation to small bowel resection in rats (Koruda et al., 1988; Chapters 2 and 3). We have recently reported that SCFA- supplemented TPN in the rat resection model halts skeletal muscle wasting as evidenced by reduced muscle atrophy, decreased *in vitro* degradation rate and reduced urine 3-methylhistidine excretion (Chapter 5). In addition, SCFA supplementation prevented abnormal hematological profiles and enhances natural killer suggesting improvements in the innate immune system (Chapter 4). The purpose of this study is to determine if SCFAs directly modulate natural killer cell cytotoxicity and skeletal muscle turnover, or if the activation of secondary signals is implied.

MATERIALS AND METHODS

Experiment #1

Experimental Model

Twelve adult male Sprague-Dawley rats (weighing 267 ± 3 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were maintained on *ad libitum* water and a non-purified diet (Rodent Laboratory Diet PMI 5001, PMI Feeds, St. Louis, MO). All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care. On the experimental day, animals were weighed and anesthetized with halothane. Animals were killed by ensanguination followed by cervical dislocation. The spleen was removed under sterile conditions and weighed. Epitrochlearis muscles were surgically removed from each foreleg and weighed.

Preparation of Splenocytes

Splenocytes were isolated under sterile conditions as previously described (Field et al., 1990) in Krebs-Ringer HEPES buffer with bovine serum albumin (5 g/L). Cells were prepared and randomly divided and assigned to be cultured in standard media (RPMI with fetal calf serum (50 g/L), 2.5 μmol/L 2-mercaptoethanol, 4000 μmol/L glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL), and (HEPES 25 mmol/L)) or the standard media including 3 m*M* SCFA (1.8 m*M* acetate, 0.75 m*M* propionate, and 0.45 m*M* butyrate). Cell viability was assessed using trypan blue exclusion and was not less than 95% for all groups.

NK Cell Cytotoxicity

NK cell cytotoxicity was determined on splenocytes in the presence or absence of SCFAs using a 4-hour chromium release assay (Field, 1995). NK-sensitive YAC-1 cells were incubated with sodium chromate (^{51}Cr, 5.55 Mbq) and seeded into 96-well v-bottom microtiter plates. Splenocytes were added in triplicate to the wells to achieve effector:target ratios between 2:1 and 100:1. After a 4-hour incubation at 37°C, an aliquot of the supernatant was counted in a gamma counter (Gamma 8000, Bechman Instruments, Inc) to determine the extent of target cell lysis. Spontaneous release was determined from target cells incubated in the absence of effector cells. Maximum release was determined from detergent lysis of labeled target cells. Cytotoxicity was calculated as follows: % specific lysis =100 x (experimental release - spontaneous release)/(maximum release - spontaneous release).

In Vitro Measurement of Protein Turnover

Methods of protein turnover have been described in detail elsewhere (Tischler et al., 1982; Strelkov et al., 1989). Muscles were weighed and preincubated in individual flasks containing 3 mL of a modified Krebs Ringer Bicarbonate medium (KRB) composed of 119 m*M* NaCl, 4.82 m*M* KCl, 1.25 m*M* $MgSO_4$, 1.0 m*M* $CaCl_2$, 1.24 m*M* NaH_2PO_4, 25 m*M* $NaHCO_3$, 2.0 m*M* N-2-hydroxyethylpiperazine-N'-N-2-ethane sulfonic acid (HEPES)/NaOH (pH 7.4), 5 m*M* glucose, 0.1 U/mL bovine pancreatic insulin and a complete mixture of amino acids present at concentrations in normal rat plasma, except tyrosine which was present at 1 nmol/mL. One muscle from each animal was incubated in the standard media outlined above, whereas the contralateral muscle was incubated in the same media containing 3 m*M* SCFA (1.8 m*M* acetate, 0.75 m*M* propionate, and 0.45 m*M* butyrate). After 30 minutes, muscles were transferred to fresh medium containing phenylalanine (1.0 m*M*) and ^{3}H-phenylalanine (0.05 μCi/mL) for a period of 3 hours. All tissues were incubated at 35°C, in media equilibrated with 95% O_2/5% CO_2. After incubation, media and muscle tissue were stored at -70°C for future determination of protein synthesis and net protein degradation. Protein synthesis was estimated by quantifying the amount of phenylalanine incorporated into trichloroacetic acid precipitated proteins. Net protein degradation rate was assessed by quantifying the net tyrosine release as this amino acid is neither synthesized nor degraded by the tissue. Isolated muscle preparations are in negative nitrogen balance *in vitro*, however changes observed in this system likely reflect qualitatively that which occurs *in vivo*. For example, Strelkov and associates (1989) using an *in vitro* approach and Tessitore and associates (1993) using an *in vivo* approach observed a similar degree of stimulation of muscle protein degradation in rats bearing the Yoshida ascites hepatoma.

Experiment #2

Animals

Forty-six adult male Sprague-Dawley rats (weighing 236 ± 7 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet (Marsman and McBurney, 1995) and drinking water. The elemental diet was given to minimize residual dietary fiber fermentation and subsequent SCFA production in the gastrointestinal tract prior to the onset of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care.

Surgical Model

Prior to surgery, animals underwent a 12 hour food restriction, were weighed and anesthetized with halothane. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (1981). Subsequently, all animals were subjected to an 80% proximal jejunoileal resection as previously described (Chapter 2).

Postoperative Care and Nutrient Solutions

Postoperatively, animals were randomly assigned to receive either standard TPN or TPN supplemented with SCFAs. Within these two diet groups, animals were further randomized to receive the parenteral solutions for either 3 or 7 days. The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain optimal sterile conditions and were filter sterilized before infusion (0.22μm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemicals, St. Louis, MO) in the concentrations used by Koruda and associates (1988) and the molar proportions found physiologically in the colon (Cummings, 1984). The diets were infused daily to provide 205 nonprotein kJ and 425 mg nitrogen. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue Preparation

On the third (TPN-3, n=12; SCFA-3, n=12) and seventh (TPN-7, n=12; SCFA-7, n=12) postoperative days, animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of it's mesenteric fat, rinsed in ice-cold saline and weighed. A 5-cm section of tissue, located 2-cm distal to the anastomosis, was removed, snap frozen in liquid nitrogen and stored at -72°C for subsequent RNA isolation and quantification.

Northern Analysis

Total RNA was isolated and quantified according to the method of Chomzynski and Sacchi (1987). RNA was electrophoresed on denaturing 1% (w/v) agarose/0.66 *M* formaldehyde gels and transferred to MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA) by capillary diffusion. The integrity and relative amounts of RNA were assessed by ultraviolet light visualization of ethidium bromide-stained RNA.

The plasmic containing the rat IL-1β cDNA was kindly provided by Dr. P.K. Lund of the University of North Carolina at Chapel Hill, North Carolina. Membranes were prehybridized for 2 hours at 50°C in a medium containing 60% (v/v) formamide, 1 x SSPE (0.18 *M* NaCl, 0.01 *M* sodium phosphate at pH 7.4, 1 m*M* EDTA), 0.5% (w/v) blotto, 10% (w/v) dextran sulfate, 1% (w/v) sodium dodecyl sulfate and 500 µg/mL salmon testes DNA. Following prehybridization, hybridization was carried out for 16-18 hours at 50°C in fresh buffer containing the [^{32}P]-labelled IL-1β riboprobe (10^6 cpm/mL). Membranes were then rinsed briefly in 2 x SSC (1 x SSC is 0.15 *M* NaCl, 0.015 *M* sodium citrate at pH 7.0), washed for 15 minutes at room temperature in 2 x SSC containing 0.1% sodium dodecyl sulfate, washed at 70°C in 0.2 x SSC containing 1% sodium dodecyl sulfate and rinsed briefly in 0.2 x SSC. The washed membranes were exposed to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (DuPont Canada, Mississauga, ON) at -70°C.

Statistics

The effect of SCFA on NK cell activity was analyzed using a split-plot one-way ANOVA. Protein synthesis and degradation was analyzed a using one-way ANOVA. The effect of SCFA on ileal IL-1β mRNA abundance was calculated using a randomized block ANOVA. Sources of variation were blots (n=3), diet (n=2), time (n=2) and diet interacted with time. Comparisons within the diet by time interaction were made by using student's t-test. All computations were done using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC). Statistical significance was defined as $p \leq 0.05$. All data are presented as mean ± SEM.

RESULTS

Experiment #1

NK Cell Cytotoxicity

The addition of SCFAs to the incubation media did not influence NK cell cytotoxicity. The percent specific lysis was similar (Figure 6.1) in splenocytes irrespective of SCFA supplementation.

In Vitro Protein Turnover

The rate of protein synthesis or net protein degradation was not altered in the presence of SCFAs. The incorporation of ^{3}H-phenylalanine into muscle protein, a measure of protein synthesis, did not differ between groups (Table 6.1). Similarly, net protein degradation, estimated by measuring tyrosine release, was not different between groups with either media (Table 6.1).

Experiment #2

IL-1β mRNA Abundance

Figure 6.2 illustrates the effect of SCFA-supplemented TPN on ileal IL-1β mRNA abundance 3 and 7 days following 80% jejunoileal resection. Ileal IL-1β mRNA was more abundant ($p<0.04$) in the 7 day SCFA-supplemented TPN group when compared to all other groups.

DISCUSSION

The results from this study do not suggest a direct effect of SCFAs on skeletal muscle protein turnover or NK cell cytotoxicity thus the *in vivo* effects of systemic SCFAs (Chapters 3 and 4) are likely mediated through the activation of secondary factors. Recently, it has become increasingly evident that cells of the gastrointestinal tract secrete a spectrum of cytokines in response to a variety of factors (Blennerhassett et al., 1992; Owens and Grisham, 1993; Eckmann et al., 1993a, b; Schuerer-Maly et al., 1994; McGowan et al., 1994; Khan and Collins, 1994; Khan et al., 1995; Jung et al., 1995). Cytokines represent a varied group of low-molecular-weight soluble factors, including interferons, interleukins, and tumour necrosis factor. Together they form a complex network of signals regulating the growth, differentiation and function of almost every cell type. Though cytokines are more critically implicated in the pathophysiologic sequelae of immune and nonimmune mechanisms of injury, their effects contribute to normal cellular homeostasis. IL-1 is a cytokine with multiple biological activities that is produced and acts on essentially all tissues of the body (Dinarello, 1991). Two forms of IL-1 are produced, IL-1α and IL-1β. Both exert identical and extremely broad biological effects, including immunologic upregulation (T- and B-cell activation, synergism with other cytokines, and cytokine gene expression) and proinflammatory activity (induction of fever and anorexia, cardiovascular suppression, neutrophilia, induction of arachidonic acid metabolites, synthesis of collagenases, osteoblast activation, etc.). IL-1 plays a major role in mediating the inflammatory response in the acute phase reaction which occurs following sepsis and trauma (Movat, 1987; Nathan, 1987; Old, 1985). Recently, Il-1 has been reported to be an accessory factor than can enhance IL-2-dependent NK cell proliferation (Robertson et al., 1993; Knoblock and Canning, 1992; Fujiwara and Grimm, 1992) suggesting a potential mechanism for the *in vivo* SCFA effect.

Following surgical trauma, the cells of the gastrointestinal tract may act to signal the innate immune system of changes in mucosal barrier function and pending bacterial translocation through the release of proinflammatory cytokines, such as IL-1. In other words, the epithelium may not function as only a mechanical barrier, but may also recognize potential pathogens and signal immune effector cells. This study provides evidence that SCFAs *per se* do not influence NK cell cytotoxicity and skeletal muscle protein turnover. We suggest that the injured, atrophied gastrointestinal tract of animals receiving standard TPN does not secrete adequate amounts of proinflammatory cytokines, such as IL-1, to stimulate immune cell recruitment and combat invasive pathogens. The results of impaired gastrointestinal defense and increased bacterial translocation may result in systemic immune cell activation and increased skeletal muscle catabolism.

Table 6.1

***Effect of SCFAs on Protein Turnover in Isolated Epitrochlearis Muscle*[1]**

	-SCFA	**+SCFA**	p value
tyrosine release (pmol tyr/mg muscle/3 hr)	134.6±7.0	133.1±7.0	NS
phenylalanine incorporation (pmol phe/mg muscle/3 hr)	105.5±4.4	104.2±4.4	NS

[1] Values are means ± SEM.

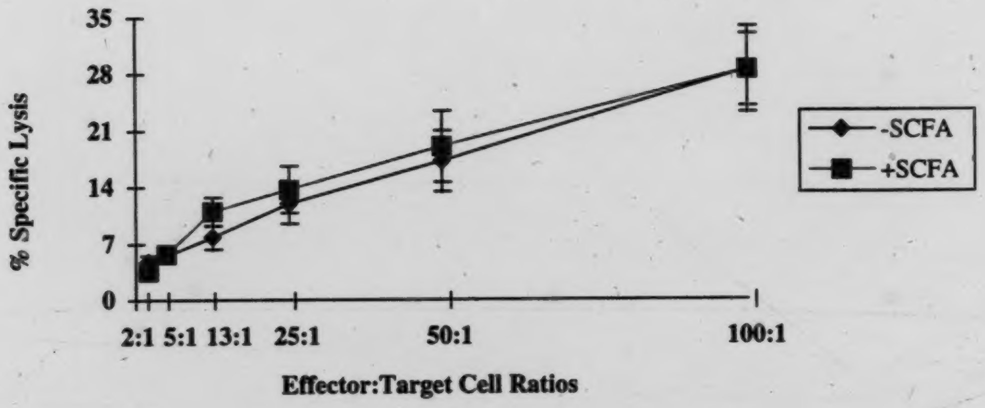

Figure 6.1 The Effect of Short-Chain Fatty Acids on Splenocyte Natural Killer Cell Cytotoxic Activity.

NK cytotoxic activity is expressed as % specific lysis = 100 x (experimental release of ^{51}Cr from ^{51}Cr-labeled YAC-1 cells - spontaneous release)/(maximum release - spontaneous release). Points represent % specific lysis at each effector-target ratio. Values are means ± SEM (-SCFA, n=12; +SCFA, n=12). There was no significant difference between the experimental groups.

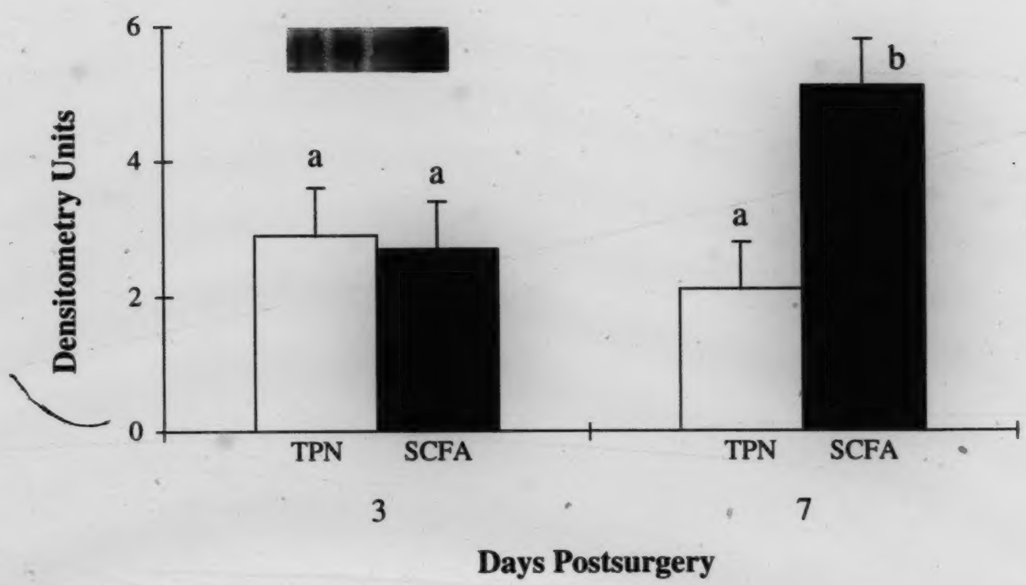

Figure 6.2 The Effect of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Ileal IL-1β mRNA Abundance 3 And 7 Days Following Intestinal Resection.

Values are means ± SEM. Ileal IL-1β mRNA was more abundant ($p<0.04$) in the 7 day SCFA-supplemented TPN group (SCFA-7, n=12) when compared to all other groups (TPN-3, n=12; TPN-7, n=12; SCFA-7, n=12). Bars that do not share a common superscript are significantly different as determined by a two-way ANOVA and student's t-test.

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CHAPTER 7

INTESTINAL STRUCTURE AND FUNCTION FOLLOWING SHORT PERIODS OF SHORT-CHAIN FATTY ACID SUPPLEMENTATION IN RATS RECEIVING TOTAL PARENTERAL NUTRITION [11]

INTRODUCTION

Since it's inception in the late 1960's, total parenteral nutrition (TPN) has become a powerful therapy used to prevent malnutrition in patients unable to absorb nutrients provided enterally. It is believed that TPN is the most important factor responsible for prolonging the lives of patients with short bowel syndrome (Purdum and Kirby, 1991; Tilson, 1980, Caniano et al., 1989). An increase in the survival of children with less than 40-cm of small intestine from 42% before 1980 to 94% in the decade since has been documented (Goulet et al., 1991). Despite these successes with TPN, when luminal nutrients are absent, intestinal atrophy and impaired function activity occur (Johnson et al., 1975; Levine et al., 1974; Feldman et al., 1976; Morin et al., 1978; Castillo et al., 1991; Inoue et al., 1993; Miura et al., 1992). Investigations aimed at identifying regulatory factors of intestinal adaptation will lead to revised TPN formulations capable of stimulating enteroplastic changes and the stable transition to total enteral nutrition.

Short chain fatty acids (SCFAs) are the byproducts of dietary fiber fermentation in the colon. Acetate, proprionate and butyrate account for approximately 85% of SCFAs and are produced intraluminally in a nearly constant molar ratio of 60:25:15 (Cummings,

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[11] A version of this chapter has been submitted for publication. Tappenden KA, Drozdowski LA, Thomson ABR, McBurney MI. Am J Clin Nutr, December, 1996.

1984). One week of SCFA supplementation has been shown to retard TPN-associated atrophy in rats with intact bowels (Koruda et al., 1990) and following 80% intestinal resection (Koruda et al., 1988). Recently, our lab reported that SCFA-supplemented TPN enhanced both structural (Chapter 2) and functional (Chapter 3) adaptation to 80% intestinal resection as early as 3 days following surgery. However, the acute effects (< 72 hours) of SCFAs on intestinal structure and function are unknown.

SCFAs may influence small intestinal mucosal proliferation by stimulating secretion of proglucagon-derived peptides. Intestinal proglucagon mRNA abundance and plasma levels of proglucagon-derived peptides are strongly correlated with cellular proliferation during intestinal adaptation (Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992; Taylor et al., 1990). Precise physiological roles for each of the proglucagon-derived peptides continue to be elucidated, however it has recently been shown that glucagon-like peptide-2 (GLP-2) modulates basolateral membrane glucose transport in the rat (Cheeseman and Tsang, 1996). We have previously reported that SCFA-supplemented TPN increases proglucagon gene expression 3 and 7 days following intestinal resection (Chapter 2). Additional information is needed on intestinal adaptation following initiation of SCFA administration in normal unresected rats. The purpose of this study is to examine factors associated with SCFA-induced enteroplasticity in the normal, unresected small intestine. Our goal is to provide potential mechanism(s) by which SCFA supplementation protects against TPN-induced atrophy.

MATERIALS AND METHODS

Animals

Thirty-one adult male Sprague-Dawley rats (weighing 258±3 g), obtained form Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet (Marsman and McBurney, 1995) and drinking water. The elemental diet was given to minimize the effect of residual dietary fiber fermentation and therefore decrease SCFA production in the gastrointestinal tract prior to the onset of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care.

Experimental Model

Prior to surgery, animals underwent a 12 hour food restriction, were weighed and anesthetized with halothane. Animals underwent superior vena cava cannulations and swivel placement as outlined by Popp and Brennan (1981). Sterile instruments and aseptic technique were used at all times. Postoperatively, animals were randomly assigned to receive either standard TPN or an isoenergetic, isonitrogenous TPN supplemented with SCFAs (TPN+SCFA). Within these two diet groups, animals were further randomized to receive the parenteral solutions for either 24 or 72 hours. The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain optimal sterile conditions and were filter sterilized before infusion (0.22 µm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemical Co., St. Louis, MO) in the molar proportions found physiologically in the colon (Cummings, 1984). The diets were infused daily to provide 217 kJ and 425 mg nitrogen. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue Preparation

On the 24th (TPN-24, n=8; TPN+SCFA-24, n=8) and 72nd (TPN-72, n=7; TPN+SCFA-72, n=8) postoperative hours, animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of it's mesenteric fat, rinsed in ice-cold saline and weighed. The intestine was divided into 3 segments with the most proximal and distal segments representing the jejunum and ileum respectively. Measuring from the ligament of Treitz or ileocaecal valve, a 2-cm section of tissue was removed, snap frozen in liquid nitrogen and stored at -70°C for subsequent RNA isolation. A 1-cm segment was stored at -70°C until assayed for DNA (Prasad et al., 1972) and protein (Lowry et al., 1951). A 15-cm segment of tissue was removed for intestinal glucose uptake studies and mucosal weight determination. Finally, a 10-cm segment was removed and mucosal scrapings were obtained for isolation of brush border and basolateral membranes.

RNA Isolation and Northern Blot Analysis

Total cellular RNA was extracted using the supplier's instructions for the Trizol™ reagent (Gibco BRL, Burlington, ON). Total cellular RNA (20 μg/lane) was size fractionated on a 1% (w/v) agarose/0.66 *M* formaldehyde gel and transferred to MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA) by capillary diffusion. The RNA was fixed to the membranes by baking *in vacuo* at 80°C for 2 hours.

The proglucagon, GLUT2 and SGLT-1 cDNA probes were labeled with [α-^{32}P]-dCTP (3000 Ci/mmol, Amersham Canada, Oakville, ON) by nick translation (Random Primers DNA Labeling System, Life Technologies, Burlington, ON). Following a 2 hour prehybridization at 65°C in hybridization buffer [6 x SSPE (0.18 *M* NaCl, 0.01 *M* sodium phosphate, 1 m*M* EDTA, pH 7.4), 0.1% SDS (wt/vol), 5 x Denhart's solution (0.5g Ficoll 400, 0.5g PVP, 0.5g bovine serum albumin], blots were incubated overnight at 68°C (45°C for SGLT-1) with fresh hybridization buffer containing the ^{32}P-labeled DNA probes. Blots were washed with 3 changes of 2 x SSPE, 0.1% SDS (wt/vol) for 20 minutes at room temperature, followed by a final wash in 0.1 x SSC, 0.1% SDS (wt/vol) for 20 minutes at 65°C. The blots were exposed to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (Dupont Canada, Mississauga, ON) at -70°C.

Relative mRNA concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, ON). The 28S ribosomal units were quantified from photographs of the ethidium bromide stained membranes prior to hybridization. These values were used to compensate for any loading discrepancies, variations in RNA integrity or nonspecific changes in RNA abundance.

The proglucagon cDNA probe, which detects a 1.2 kb proglucagon transcript, was a gift from Peter J Fuller of Prince Henry's Institute of Medical Research in Melbourne, Australia. The GLUT2 plasmid was donated by Dr. G.I. Bell of Howard Hughes Medical Institute, University of Chicago and detects a 3.8 kb GLUT2 species. Dr. N. Davidson (University of Chicago, IL) supplied the SGLT-1 plasmid which detects a 4.8 kb SGLT-1 transcript.

Brush Border and Basolateral Membrane Isolation

This method was derived from a combination of procedures previously published (Maenz and Cheeseman, 1986; Orsenigo et al., 1985; Yakymyshyn et al., 1982) for the simultaneous isolation of basolateral and brush border membranes. Mucosal scrapings were homogenized in sucrose-tris buffer (250 m*M* sucrose - 2 m*M* Tris-HCl - 0.1 m*M* phenyl methyl sulfonyl fluoride buffer, pH 7.4) three times for 30 seconds using a Polytron at setting 8 (Brinkman). The homogenate was centrifuged 15 minutes at 2400 x g with no brake. The pellet (unbroken cells, nuclear material) was discarded, and the supernatant was centrifuged 20 minutes at 43 700 x g with no brake. The resulting supernatant was discarded. The upper white fluffy pellet (P1) was resuspended in sucrose-tris buffer. The lower dark pellet (P2) was resuspended in sucrose-tris buffer and centrifuged 20 minutes at 43700 x g with no brake. The supernatant was discarded and the upper white fluffy pellet resuspended in sucrose-tris buffer and combined with P1. The lower dark pellet was resuspended in sucrose-tris buffer and combined with the P2.

The combined P1 suspension was homogenized 15 seconds at setting 8, layered onto a 20% Percoll™ gradient, and centrifuged 30 minutes at 46 000 x g with maximum brake. The resulting white fluffy basolateral membrane layer was then centrifuged 30 minutes at

115 000 x g with maximum brake. The membrane layer was removed, resuspended in sucrose-tris buffer, and homogenized 15 seconds at setting 8. 1 *M* CaCl2 was added to a final concentration of 10 m*M* and the homogenate was stirred gently on ice for 10 minutes. The mixture was then centrifuged 10 minutes at 7700 x g. The supernatant was discarded and the pellet resuspended in sucrose-tris buffer. The resuspended pellet was then homogenized for 15 seconds at setting 8 and centrifuged 20 minutes at 46 000 x g. The supernatant was discarded and the final basolateral membrane pellet was resuspended in sucrose-tris buffer.

The combined P2 suspension was homogenized 15 seconds at setting 8 and centrifuged 10 minutes at 1900 x g. The resulting pellet was discarded and the supernatant was centrifuged 15 minutes at 14 600 x g. The pellet was discarded. 1 *M* $CaCl_2$ was added to the supernatant to a final concentration of 10 m*M* and the homogenate was gently stirred on ice for 20 minutes. The mixture was centrifuged 10 minutes at 3000 x g with maximum brake. The supernatant was then centrifuged 30 minutes at 46000 x g, and the resulting final brush border membrane pellet was resuspended in distilled deionized water. Membrane purity was confirmed through 10-20 fold enrichment of brush border and basolateral membrane markers, alkaline phosphatase and Na^+/K^+, ATPase respectively. Aliquots were stored at -80°C.

Western Blot Analysis

Brush border and basolateral membrane proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel elecrophoresis using the Mini-Protean II electrophoresis system (BioRad Laboratories (Canada) Ltd., Mississauga, ON) and then transferred onto MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA). The blotting conditions were optimized by multiple trials to ensure complete protein transfer from gel to membrane. After blotting the membrane was stained with Amido Black to ensure homogenous protein transfer and the gel was stained with Commassie Blue to document complete protein transfer. Primary antibodies (Oncogene Science Inc., Uniondale, NY) included anti-GLUT2 purified rabbit polyclonal IgG antibody (GLUT2 molecular weight, 60 kDa) and anti-SGLT-1 purified rabbit polyclonal IgG antibody (SGLT-1 molecular weight, 73 kDa). Immunoblotting was conducted according to the manufacturers instructions. Briefly, nonspecific binding sites were blocked by immersing the membrane in 5% nonfat dry milk in Tris-buffered saline with Tween 20 (TBST: composed of 20 mmol/L Tris, 137 mmol/L NaCl, 0.05% Tween 20, pH 7.6) for 1 hour at room temperature on a metabolic shaker. The membrane was then washed 3 x 10 minutes in TBST at room temperature. Primary antibodies were diluted 1:500 in TBST and incubated with membranes for 4 hours at room temperature. Blots were washed 3 x 10 minutes at room temperature and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG immunoglobulin (Pierce, Rockford, IL) at 1:2000 dilution in TBST for 1 hour at room temperature. Blots were washed again 3 x 10 minutes as described above. Positive fluorescence reaction was developed using the SuperSignal™ CL-HRP Substrate System (Pierce, Rockford, IL) and exposed to enhanced chemiluminescence detection Hyperfilm™-ECL (Amersham, Oakville, ON). Relative protein concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, ON).

Measurement of Transport Kinetics

Determination of transport kinetics was done as previously described (Thomson and Rajotte, 1983a). Briefly, the 15-cm segment of jejunum and ileum removed from each animal was opened along it's mesenteric border and the mucosal surface was carefully washed with cold saline to remove visible mucus and debris. Pieces of intestine (1-cm^2) were cut out and the tissue was mounted as flat sheets in incubation chambers containing oxygenated Kreb's bicarbonate buffer (pH 7.4) at 37°C. Tissue discs were preincubated in this buffer for 15 minutes to allow equilibration at this temperature. After preincubation, the chambers were transferred for 6 minutes to other beakers containing [^{3}H]-inulin and various [^{14}C]-probe molecules in oxygenated Krebs bicarbonate (pH 7.4 and 37°C). The concentration of solutes was: lauric acid (12:0), 0.1 m*M*; *D*-fructose, 4, 8, 16, 32 or 64 m*M*; *D*-glucose, 4, 8, 16, 32, or 64 m*M*; and *L*-glucose, 1 or 16 m*M*. The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars, and the stirring rates were precisely adjusted by means of a strobe light. A stirring rate of 600 rpm was selected to achieve low effective resistance of the intestinal unstirred water (Thomson and Dietschy, 1980). The experiment was terminated by removing the chambers and quickly rinsing the tissue in cold saline for approximately 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch. For all probes the tissue was dried overnight in an oven at 55°C . The dry weight of the tissue was determined, the sample was saponified with 0.75 *N* NaOH, scintillation fluid was added (Beckman Ready Solv HP), and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes. The mucosal weight was determined following scraping of the intestine from adjacent samples not used for uptake studies. The weight of the mucosa in the samples used to measure uptake was determined by multiplying the dry weight of the intestinal sample by the percentage of the intestinal wall comprised of mucosa. As the proportion of mucosa in the total jejunum or ileum did not differ between groups, the uptake of nutrients was expressed as nmol•100 mg intestine^{-1}•minute^{-1}.

Glucagon-Like Peptide-2 Radioimmunoassay

Blood was collected by cardiac puncture into a prechilled syringe containing EDTA (1 mg/mL blood, Sigma Chemical Co., St. Louis, MO) and aprotinin (500 KIU/mL blood, Sigma Chemical Co., St. Louis, MO). Plasma was separated by centrifugation and stored at -70°C for subsequent GLP-2 determination. Plasma GLP-2 concentration was quantified using a commercially available kit (Peninsula Laboratories, Inc., Belmont, CA). Briefly, peptides were extracted from plasma using C_{18} SEP-COLUMNS (Peninsula Laboratories, Inc., Belmont, CA), 0.1% trifluoroacetic acid and 60% acetonitrile as elution solvents (Peninsula Laboratories, Inc., Belmont, CA). Concentrations of GLP-2 were measured using a competitive ^{125}I-GLP-2 binding assay. This kit measures GLP-2 with 0% crossreactivity with the various GLP-1 forms. The IC_{50} for this assay is 108 pg/tube at a binding of 98.9% (defined as the mean total bound minus 2 SD from the mean total bound). The intra-assay coefficient of variance was 1.25%.

Statistics

All data are expressed as mean ± SEM. Differences between treatments were determined using a randomized block ANOVA. Sources of variation were blocks (surgery day = 8), diet (diet = 2), time (time = 2) and diet interacted with time. Computations within the diet by time interaction were made using student's t-test. Computations were done using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC). Statistical significance was defined as $p \leq 0.05$.

RESULTS

Nutritional Support

All animals received continuous nutrient infusion throughout the study period. Nutrient solutions were infused at a rate previously established to support growth comparable to nonsurgical, *ab libitum* chow fed controls (Chapter 2).

Jejunal and Ileal Characteristics

SCFA-supplemented TPN had no influence on the intestinal weight (mg/cm) or percentage of the intestinal wall comprised of the mucosa in the jejunum or ileum at either time point (Table 7.1). In the jejunum, protein, DNA and RNA concentrations did not differ between groups (Table 7.1). However, ileal protein concentration was reduced ($p<0.05$) in both the 24 and 72 hour TPN+SCFA groups when compared to the control TPN groups (Table 7.1). Ileal DNA concentration significantly increased ($p<0.05$) between the 24 and 72 hour TPN+SCFA groups however no change was noted within the TPN control groups (Table 7.1). Ileal RNA concentration was significantly increased ($p<0.05$) in the TPN+SCFA groups, however the ileal RNA concentration declined ($p<0.03$) with time irrespective of diet (Table 7.1).

GLUT2 and SGLT-1 mRNA and Protein Abundance

GLUT2 mRNA abundance was significantly higher ($p=0.03$) in the TPN+SCFA groups at both time points when compared to the control TPN groups (Figure 7.1). The GLUT2 protein abundance within the jejunal basolateral membrane did not differ between groups (Figure 7.2). However, in the ileal basolateral membrane, the abundance of the GLUT2 protein was significantly higher ($p<0.04$) following 72 hours of SCFA-supplemented TPN compared to all other groups (Figure 7.2). Ileal GLUT2 protein abundance in the 72 hour TPN+SCFA group ileum was significantly greater ($p<0.05$) than that of the jejunum.

The SGLT-1 mRNA abundance in jejunal total cellular RNA did not differ among groups (Table 7.2). Similarly, the SGLT-1 protein abundance in the jejunal and ileal brush border membrane was not altered by SCFA-supplemented TPN following 24 or 72 hours of infusion (Table 7.2). Following 72 hours of TPN+SCFA, the abundance of SGLT-1 protein was significantly greater ($p<0.05$) in the ileum than the jejunum (Table 7.2).

In Vitro Nutrient Uptake

Jejunal and ileal uptake of 4-64 mM *D*-glucose was not altered by SCFA-supplemented TPN administration as there were no differences in the estimated values for the maximal transport rates (V_{MAX}) and apparent Michaelis affinity constant (K_M; Table 7.3). Jejunal uptake of *L*-glucose, used to estimate the apparent passive permeability coefficient, was significantly higher following 72 hours of TPN+SCFA when compared to the 24 hour TPN+SCFA group (Table 7.4). Likewise, jejunal uptake of lauric acid, an indirect measure of unstirred water resistance, was significantly higher ($p<0.05$) in the 72 hour SCFA group when compared to the 24 hour SCFA group suggesting a lower unstirred water layer resistance in this group (Table 7.4). The lauric acid uptake did not differ between the 24 and 72 hour control TPN groups (Table 7.4). SCFA supplemented TPN had no influence on *D*-fructose uptake in the jejunum or ileum.

Proglucagon mRNA Abundance and Plasma GLP-2 Concentration

As seen in Figure 7.3, the proglucagon mRNA abundance was significantly upregulated ($p<0.05$) following 24 hours of SCFA supplemented-TPN when compared to the control TPN groups at both time points. The 72 hour TPN+SCFA group was not significantly lower than the 24 hour TPN+SCFA group, nor did it differ from the control TPN groups. Plasma GLP-2 concentration was not significantly altered by SCFA-supplemented TPN following 24 or 72 hours of infusion (Figure 7.4).

DISCUSSION

Intestinal adaptation has primarily been explored by resecting a portion of the small intestine (Wilmore et al., 1971) and monitoring the morphological and functional reactions to the intestinal insult. SCFAs have been shown to promote intestinal adaptation in this surgical model within 7 days of administration (Koruda et al., 1988; Chapters 2 and 3), however fewer studies have examined the enteroplastic responses to SCFA-supplemented TPN in the normal, unresected gut (Koruda et al., 1990). TPN therapy induces atrophy in patients with an intact small intestine thus information regarding the effect of gut specific fuels in this physiologically discreet, yet prevalent clinical scenario is lacking. This experiment begins to elucidate the early effects of SCFAs on the intestine in normal rats. SCFA supplementation rapidly increased ileal DNA and RNA content, jejunal GLUT2 mRNA, ileal GLUT2 protein and ileal proglucagon mRNA.

During the first 72 hours of SCFA supplementation, no changes in protein, DNA or RNA concentration occurred in the jejunum. Early responses to systemic SCFAs in the ileum include decreased ileal protein and increased DNA and RNA contents, which may reflect an increased proportion of cells undergoing mitosis which are functionally immature (Menge et al., 1982).

Systemic SCFAs do not appear to influence the brush border sodium/glucose co-transporter, SGLT-1. The upregulation of jejunal GLUT2 gene expression with SCFA supplementation is consistent with previous reports from our lab (Chapter 3). Increased ileal GLUT2 protein abundance following 72 hours of SCFA supplemented TPN suggests that this message is translated into protein and inserted into the basolateral membrane.

The lack of response of jejunal and ileal glucose uptake to systemic SCFAs was unexpected since ileal D-glucose uptake increased with SCFA supplementation for 3 and 7 days following an 80% intestinal resection (Chapter 3). It appears that an intestinal insult may be required as trophic stimuli for measurable SCFA-induced changes in glucose uptake to occur within 3 days. In contrast to the short administration of parenteral nutrients presented herein, many studies showing increased glucose uptake involve overt models of intestinal adaptation such as intestinal resection (Gleeson et al., 1972; Robinson et al., 1982; Thomson, 1986), diabetes (Fedorak et al., 1987; Olsen and Rogers, 1970; Thomson, 1980, 1981, 1983a,b; Thomson and Rajotte, 1983a,b), pregnancy (Musacchia and Hartner, 1970) and lactation (Cripps and Williams, 1975).

The increased lauric acid (12:0) uptake, which was used as an indirect measure for unstirred water resistance, indicates that following 72 hours of SCFA supplementation, there is less unstirred water layer resistance than following 24 hours of SCFA supplementation. The increase in L-glucose uptake with time in the SCFA groups may directly reflect decreased unstirred water layer resistance or alternatively, a mechanism whereby macromolecular nutrient transport is increased. Gut mucosal macromolecular transmission is believed to be an important phenomena associated with TPN in response to shortage of substrate (Koshibuchi et al., 1995).

The manner in which SCFAs induce adaptive changes is not directly addressed in the current study. Many studies have established a strong relationship between cellular proliferation and elevated levels of proglucagon mRNA and proglucagon derived peptides (Dowling, 1982; Bloom and Polak, 1982; Sagor et al., 1983; Gornacz et al., 1984; Al-

Mukhtar et al., 1983). SCFAs increased ileal proglucagon mRNA in normal, unresected rats following 24 hours of supplementation, however this increase was not maintained in the 72 hour SCFA+TPN group. Taylor and associates (1990) reported that following intestinal resection, proglucagon expression increased threefold, peaking two days following surgery and declining thereafter. Whereas we have recently shown that SCFA-supplemented TPN increased proglucagon mRNA abundance both 3 and 7 days following intestinal resection (Chapter 2). Within the intestinal resection model, SCFAs may exert their trophic effect by extending the duration of increased proglucagon expression. In summary, SCFA supplemented TPN rapidly upregulates ileal DNA and RNA concentration, enhances jejunal GLUT2 mRNA and ileal GLUT2 abundance, and increases ileal proglucagon mRNA. This study substantiates proglucagon involvement in intestinal adaptation however it's modulation by SCFAs are of longer duration following intestinal insult.

Table 7.1

Effect of SCFA-Supplemented TPN on Jejunal and Ileal Protein, DNA and RNA Concentrations After 24 or 72 Hours of Nutrient Infusion[1]

	TPN		TPN+SCFA		DIET	TIME
	24 hour (n=8)	**72 hour** (n=7)	**24 hour** (n=8)	**72 hour** (n=8)		
jejunal weight	8.4 ± 0.7	8.7 ± 0.9	8.8 ± 0.7	7.8 ± 0.3	NS	NS
(mg/cm)						
ileal weight	9.0 ± 0.5	7.7 ± 0.4	9.9 ± 0.8	9.3 ± 0.8	NS	NS
jejunal % mucosa	57.2 ± 3.3	56.7 ± 3.8	55.9 ± 3.7	59.1 ± 2.2	NS	NS
ileal % mucosa	55.7 ± 4.0	57.6 ± 7.3	55.8 ± 3.6	53.2 ± 3.9	NS	NS
jejunal protein	110.9 ± 5.4	103.0 ± 6.3	111.2 ± 6.9	98.2 ± 6.9	NS	NS
(µg pro/mg jejunum)						
ileal protein	106.2 ± 3.9	112.9 ± 4.4	100.1 ± 4.4	100.4 ± 4.4	$p < 0.05$	NS
jejunal DNA	$4.0 \pm 0.6^{*}$	4.1 ± 0.7	4.1 ± 0.6	$3.5 \pm 0.6^{**}$	NS	NS
(µg DNA/mg jejunum)						
ileal DNA	$6.7 \pm 0.8^{ab*}$	5.6 ± 0.9^{ab}	5.0 ± 0.9^{a}	$8.0 \pm 0.9^{b**}$	NS^2	NS^2
jejunal RNA	3.8 ± 0.2	3.5 ± 0.2	$3.7 \pm 0.2^{*}$	3.5 ± 0.2	NS	NS
(µg RNA/mg jejunum)						
ileal RNA	4.0 ± 0.3^{ab}	3.4 ± 0.4^{a}	$4.9 \pm 0.4^{b*}$	3.9 ± 0.4^{a}	$p = 0.05^2$	$p < 0.03^2$

[1] Values are means ± SEM.

[2] Indicates a significant interaction exists between diet and length ($p<0.03$). When a significant interaction exists, means within a row with different superscripts are signifantly different.

* Indicates a significant difference exists between gastrointestinal segments containing (*) within that column ($p<0.01$).

** Indicates a significant difference exists between gastrointestinal segments containing (**) within that column ($p<0.0001$).

Table 7.2

***Effect of SCFA-Supplemented TPN on SGLT-1 mRNA and Protein Abundance After 24 or 72 Hours of Nutrient Infusion*[1]**

	TPN		TPN + SCFA			
densitometry units	**24 hour** (n=4)	**72 hour** (n=4)	**24 hour** (n=4)	**72 hour** (n=4)	**DIET**	**TIME**
jejunal SGLT-1 mRNA	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	NS	NS
jejunal SGLT-1 protein	2.8 ± 1.3	0.3 ± 1.0*	2.6 ± 1.3	2.9 ± 1.0	NS	NS
ileal SGLT-1 protein	1.7 ± 0.7	2.3 ± 0.7*	2.2 ± 0.7	3.1 ± 0.7	NS	NS

[1] Values are means ± SEM.

* Indicates a significant difference exists between gastrointestinal segment containing (*) within that column (p<0.05).

Table 7.3

Effect of SCFA-Supplemented TPN on D-Glucose Uptake.[1]

	TPN		TPN + SCFA		DIET	TIME
	24 hour (n=8)	72 hour (n=7)	24 hour (n=8)	72 hour (n=8)		
Jejunum						
V_{MAX}	1196 ± 135	1047 ± 216	1181 ± 199	1284 ± 98	NS	NS
K_M	30.2 ± 6.6	19.8 ± 8.3	22.9 ± 7.9	24.1 ± 4.2	NS	NS
Ileum						
V_{MAX}	944 ± 127	1018 ± 114	857 ± 101	949 ± 99	NS	NS
K_M	17.3 ± 5.8	12.2 ± 3.4	15.1 ± 3.8	16.4 ± 3.5	NS	NS

[1] Values are means ± SEM.

Table 7.4

***Effect of SCFA-Supplemented TPN on L-Glucose and Lauric Acid Uptake.*[1]**

nmol•100 mg intestine^{-1}•minute^{-1}	**TPN**		**TPN + SCFA**		**DIET**	**TIME**
	24 hour (n=8)	**72 hour** (n=7)	**24 hour** (n=8)	**72 hour** (n=8)		
Jejunum						
1 m*M* *L*-Glucose	9.8 ± 0.8^{a}	11.9 ± 1.2^{a}	10.9 ± 0.9^{a}	14.2 ± 0.8^{b}	NS^{2}	NS^{2}
16 m*M* *L*-Glucose	157 ± 13^{a}	191 ± 20^{a}	174 ± 15^{a}	227 ± 13^{b}	NS^{2}	NS^{2}
0.1 m*M* Lauric Acid	12.8 ± 1.8^{a}	17.4 ± 2.0^{ab}	9.7 ± 1.8^{a}	16.5 ± 2.4^{b}	NS^{2}	NS^{2}
Ileum						
1 m*M* *L*-Glucose	9.5 ± 0.8	11.3 ± 1.6	10.3 ± 1.4	12.0 ± 1.2	NS	NS
16 m*M* *L*-Glucose	153 ± 20	180 ± 26	164 ± 23	192 ± 20	NS	NS
0.1 m*M* Lauric Acid	12.7 ± 1.7	16.1 ± 1.2	10.4 ± 1.3	12.9 ± 2.8	NS	NS

[1] Values are means ± SEM.

[2] Indicates a significant interaction exists between diet and length. When a significant interaction exists, means within a row with different superscripts are significantly different.

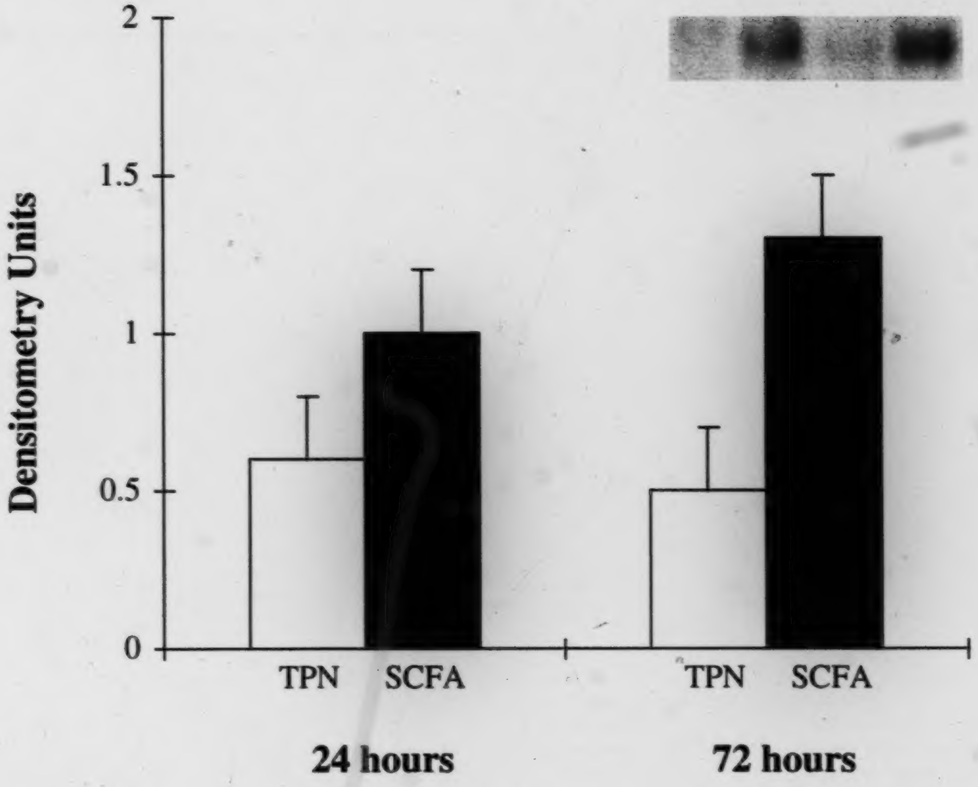

Figure 7.1 The Effect of 24 or 72 Hours of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Jejunal GLUT2 mRNA Abundance.

Values are means ± SEM. The SCFA supplemented TPN increased (p=0.03) GLUT2 mRNA abundance following 24 (TPN-24, n=8; SCFA-24, n=8) and 72 hours (TPN-72,n=7; SCFA-72, n=8) of administration.

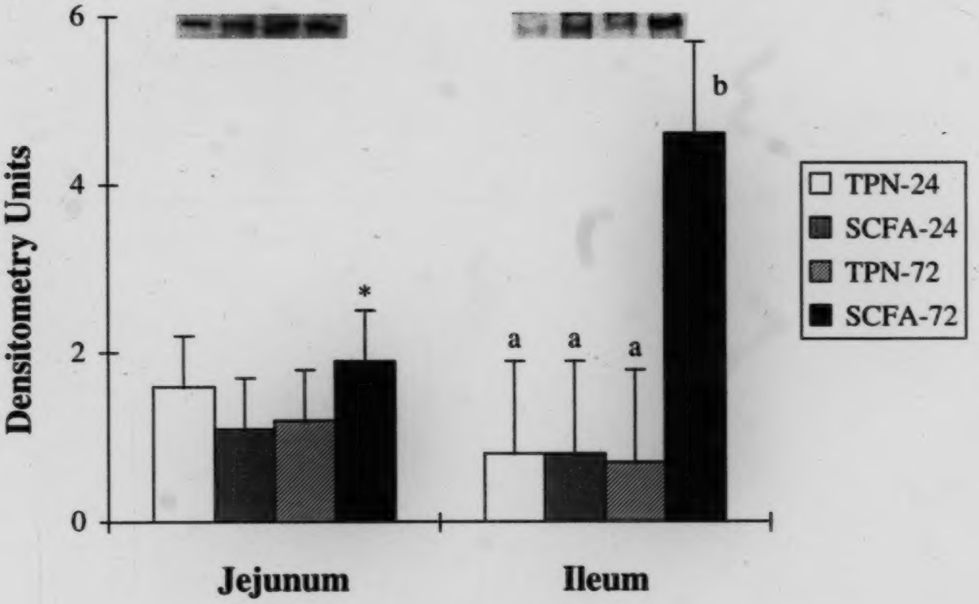

Figure 7.2 The Effect of 24 or 72 Hours of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Jejunal and Ileal GLUT2 Protein Abundance. Values are means ± SEM. Within an intestinal segment, means with different letters are different ($p<0.04$). *Indicates mean is different from ileal value for that nutrient solution and administration time. The abundance of ileal GLUT2 protein was significantly higher ($p<0.04$) following 72 hours of SCFA-supplemented TPN (SCFA-7, n=4) compared to all other groups (TPN-3, n=4; SCFA-4, n=4; SCFA-7, n=7). The abundance of jejunal GLUT2 protein did not differ between groups.

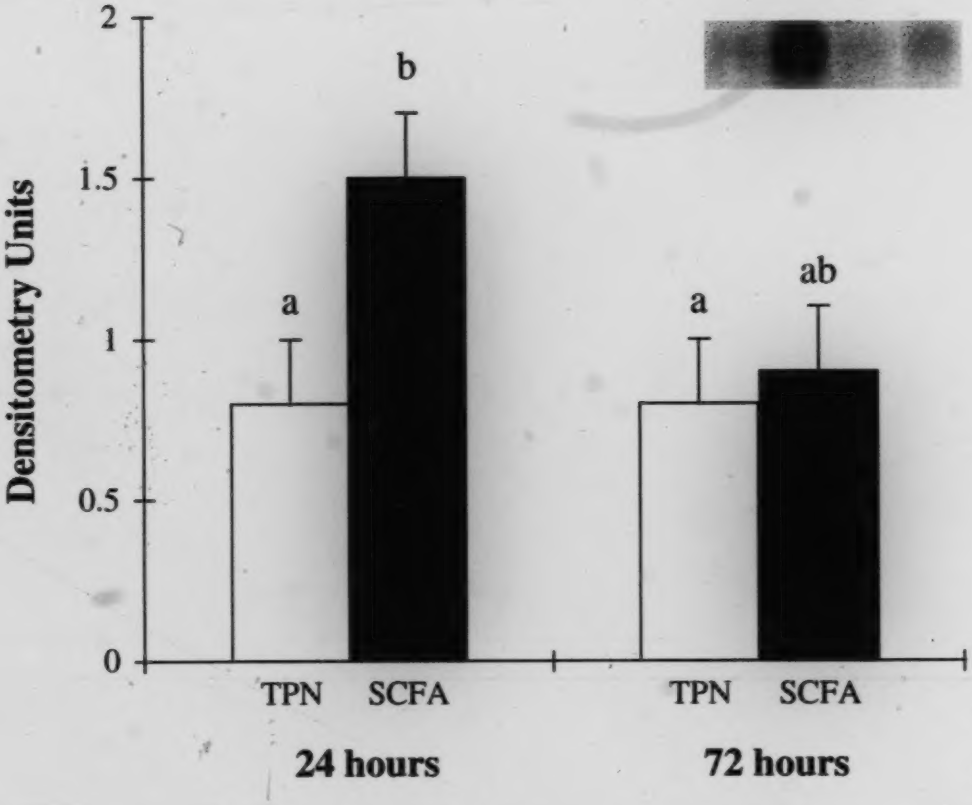

Figure 7.3 The Effect of 24 or 72 Hours of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Ileal Proglucagon mRNA Abundance.

Values are means ± SEM, n=7-8. Means with different letters are different ($p<0.05$). The proglucagon mRNA abundance was significantly upregulated ($p<0.05$) following 24 hours of SCFA-supplemented TPN when compared to the control TPN groups at both time points. Means with different letters are significantly different as identified by a two-way ANOVA and student's t-test.

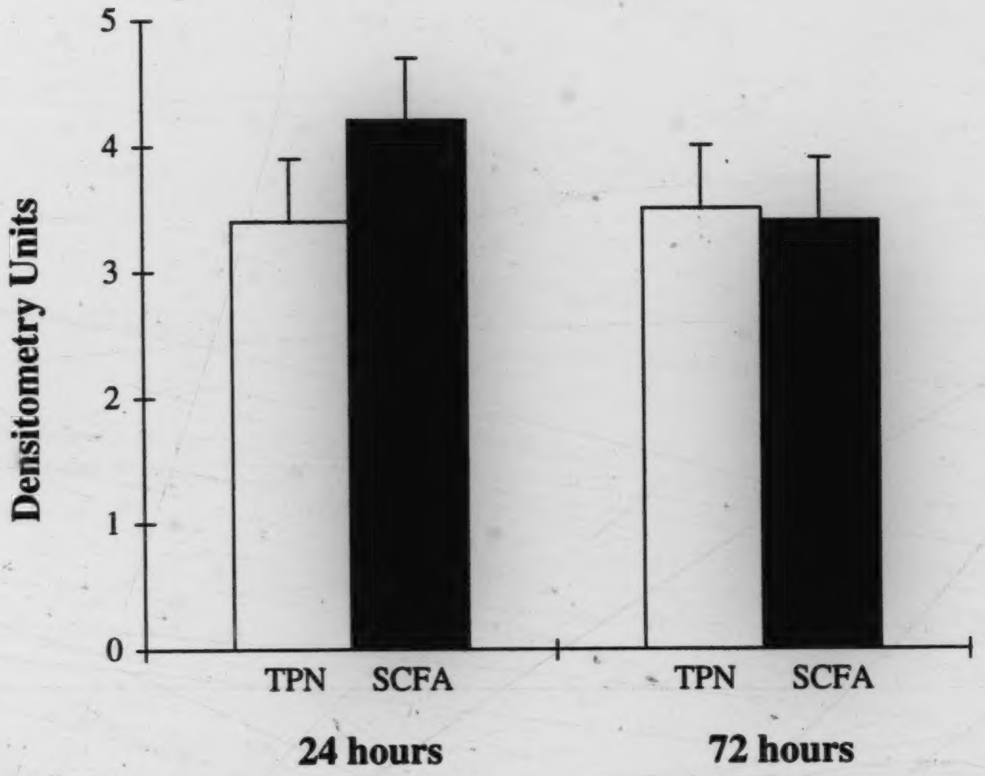

Figure 7.4 The Effect of 24 or 72 Hours of Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition on Plasma Glucagon-Like Peptide-2 Concentration. Values are means ± SEM, n=7-8. SCFA supplemented TPN did not influence plasma GLP-2 concentration following 24 or 72 hours of infusion.

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CHAPTER 8

SYSTEMIC SHORT-CHAIN FATTY ACIDS RAPIDLY UPREGULATE PROGLUCAGON, C-MYC, C-JUN AND C-FOS MESSENGER RNA ABUNDANCE [12,13]

INTRODUCTION

The epithelium of the intestinal mucosa is one of the most rapidly proliferating tissues in the body, being replaced every 2-3 days (Lipkin, 1987). Maintenance of the intestinal mucosa is highly dependent on route of nutritional support. It is well documented that total parenteral nutrition (TPN) results in mucosal atrophy (Johnson et al., 1975) and decreased functional capacity (Inoue et al., 1993). TPN-associated atrophy also occurs in the presence of trophic stimuli such as intestinal resection (Ford et al., 1984; Morin et al., 1978). TPN supplemented with short-chain fatty acids (SCFAs) partially reverses TPN-induced atrophy in normal (Koruda et al., 1990) and resected small intestine (Koruda et al., 1988; Chapters 2 and 3) but the mechanism is unclear.

Proglucagon-derived peptides originate from post-translational processing of the proglucagon gene (Mojsov et al., 1986; Orskov et al., 1987) in the enteroendocrine L-cells, found predominantly in the ileum and colon (Larsson et al., 1975). Intestinal proglucagon mRNA abundance and plasma levels of proglucagon-derived peptides are strongly correlated with cellular proliferation during intestinal adaptation (Bloom and Polak, 1982; Sagor et al., 1983; Rountree et al., 1992). Precise physiological roles for

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[12] A version of this chapter has been submitted for publication. Tappenden KA, McBurney MI. Am J Physiol, October, 1996.

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each of the proglucagon-derived peptides continue to be elucidated, however it has recently been postulated that glucagon-like peptide-2 (GLP-2) upregulates basolateral membrane glucose transport in the rat (Cheeseman and Tsang, 1996). We have previously reported that SCFA supplemented TPN increases proglucagon gene expression 3 and 7 days following intestinal resection (Chapter 2). The initial effect of SCFA supplementation to TPN on proglucagon expression and proglucagon-derived peptide abundance is unknown.

c-*myc*, c-*jun* and c-*fos* are proto-oncogenes that are involved in control of the cell cycle and proliferation and may play a role in the regulation of normal growth of the gastrointestinal mucosa. c-*myc*, the earliest discovered nuclear oncogene, encodes for a nuclear phosphoprotein that functions as a transcriptional factor controlling cell division, differentiation and apoptosis (Marcu et al., 1992). In addition, c-*myc* is involved in intracellular transduction and multiplication of growth-promoting signals from stimulated growth-factor receptors at the cell surface (Rottleb et al., 1995). c-*fos* and c-*jun* encode for DNA binding proteins that dimerize to form the AP-1 transcription factor (de Groot et al., 1990). AP-1 DNA binding regulates multiple genes involved in cellular growth and regulation (de Groot et al., 1990).

c-*fos* and c-*jun* expression is upregulated in the proliferative period that is induced by intraluminal nutrients following fasting (Hodin et al., 1994; Holt and DuBois, 1991). *In vitro* work has demonstrated that the SCFA, butyrate alters c-*myc* (Rabizadeh et al., 1993; Rottleb et al., 1995), c-*fos* (Souleimani and Asselin, 1993) and c-*jun* (Nishina et al., 1993, Rabizadeh et al., 1993) expression thereby modulating proliferation and differentiation. Until recently, butyrate was believed to lack biological effects on oncogene expression *in vivo* due to it's rapid metabolism (Miller et al., 1987). However, earlier this year Velázquez and associates (1996) reported that the injection of butyrate into the colonic lumen increases c-*Jun* protein abundance in association with changes in crypt compartment-specific proliferation in normal rat colon *in vivo*. The action of systemic butyrate on proto-oncogene expression is unknown. The purpose of this study is to examine the acute effects of SCFA-supplemented TPN on mucosal structure and function. Proglucagon expression, plasma GLP-2 concentration and changes in oncogene expression are measured to assess if rapid changes in these trophic signals may be involved in the mechanism by which SCFA prevent TPN-induced mucosal atrophy.

MATERIALS AND METHODS

Animals

Thirty-eight adult male Sprague-Dawley rats (weighing 246±2 g), obtained from Health Sciences Laboratory Animal Services (University of Alberta, Edmonton, Canada), were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Four days prior to surgery, the animals were given free access to a nutritionally complete elemental diet (Marsman and McBurney, 1995) and drinking water. The elemental diet was given to minimize dietary fiber fermentation and therefore decrease SCFA production in the gastrointestinal tract prior to the onset of the experiment. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council in Animal Care.

Experimental Model

On experimental day 0, animals were weighed and underwent superior vena cava cannulations and swivel placement (Popp and Brennan, 1981). Following recovery, all animals received continuous nutrient infusions for 72 hours as either standard TPN or an isoenergetic, isonitrogenous TPN supplemented with SCFAs. Animals were randomly assigned to 1 of 5 treatments: standard TPN for 72 hours (TPN-72, n=7); TPN+SCFA for 72 hours (SCFA-72, n=8), or; standard TPN followed by TPN+SCFA for the final 6 (SCFA-6, n=8), 12 (SCFA-12, n=7) or 24 (SCFA-24, n=8) experimental hours (Figure

8.1). The TPN solutions (Table 2.1) were prepared daily under a laminar flow hood to maintain sterile conditions and were filter sterilized before infusion (0.22 µm millipore filter, Millipore Corporation, Bedford, MA). The SCFAs - acetate, propionate and butyrate - were added as sodium salts (Sigma Chemical Co., St. Louis, MO) in the concentrations previously used by Rombeau's group (Koruda et al., 1988, 1990) and the molar proportions found physiologically in the colon (Cummings, 1984). The diets were infused daily to provide 52 kcals and 425 mg nitrogen with a nonprotein kilocalorie-to-nitrogen ratio of 116. The nutrient solutions were administered using a Harvard infusion pump (Harvard Apparatus, Wellesley, MA). All animals had free access to drinking water throughout the study period.

Tissue Preparation

Following 72 hours of nutrient infusions, all animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve, freed of it's mesenteric fat, rinsed in ice-cold saline and weighed. The intestine was divided into 3 segments with the most proximal and distal segments representing the jejunum and ileum, respectively. Within the jejunum or ileum, a 5-cm section of tissue was removed, snap frozen in liquid nitrogen and stored at -70°C for subsequent RNA isolation and quantification. A 1-cm segment was stored at -70°C until assayed for DNA (Prasad et al., 1972) and protein (Lowry et al., 1951). Finally, a 20-cm segment was removed and mucosal scrapings were obtained for isolation of brush border and basolateral membranes.

RNA Isolation and Northern Blot Analysis

Total cellular RNA was extracted using the supplier's instructions for the Trizol™ reagent (Gibco BRL, Burlington, ON). Total cellular RNA (20 µg/lane) was size fractionated on a 1% (w/v) agarose/0.66 *M* formaldehyde gel and transferred to MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA) by capillary diffusion. The RNA was fixed to the membranes by baking *in vacuo* at 80°C for 2 hours.

The proglucagon cDNA probe, a gift from Dr. P.J. Fuller (Prince Henry's Institute of Medical Research, Melbourne, Australia) and GLUT2 cDNA probe, donated by Dr. G.I. Bell (Howard Hughes Medical Institute, University of Chicago) were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham Canada, Oakville, ON) by nick translation (Random Primers DNA Labeling System, Life Technologies, Burlington, ON). Following a 2 hour prehybridization at 65°C in hybridization buffer [6 x SSPE (0.18 *M* NaCl, 0.01 *M* sodium phosphate, 1 m*M* EDTA, pH 7.4), 0.1% SDS (wt/vol), 5 x Denhart's solution (0.5 g Ficoll 400, 0.5 g PVP, 0.5 g bovine serum albumin], blots were incubated overnight at 68°C with fresh hybridization buffer containing the ^{32}P-labeled DNA probes. Blots were washed with 3 changes of 2 x SSPE, 0.1% SDS (wt/vol) for 20 minutes at room temperature, followed by a final wash in 0.1 x SSC, 0.1% SDS (wt/vol) for 20 minutes at 65°C. The blots were exposed to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (Dupont Canada, Mississauga, ON) at -70°C.

The *c-myc*, *c-jun* and *c-fos* probes were 40 base single-stranded synthetic probes supplied by Oncogene Research Products (Cambridge, MA). The 40mers were 5'-end labeled with [γ-^{32}P]ATP (ICN Biomedicals, Inc., Irving, CA) using T4 polynucleotide kinase and separated from unincorporated nucleotide. Prehybridization, hybridization and washing was performed under high stringency conditions. All procedures were performed according to the supplier's instructions.

Relative mRNA concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, ON). The 28S ribosomal units were quantified from photographs of the ethidium bromide stained membranes prior to hybridization. These values were used to compensate for any loading discrepancies, variations in RNA integrity or nonspecific changes in RNA abundance.

Enterocyte Subcellular Membrane Fractionation

This method was derived from a combination of procedures previously published (Maenz and Cheeseman, 1986; Orsenigo et al., 1985; Yakymyshyn et al., 1982) for the simultaneous isolation of basolateral, microsomal and brush border membranes. Mucosal scrapings were homogenized in sucrose-tris buffer (250 m*M* sucrose - 2 m*M* Tris-HCl - 0.1 m*M* phenyl methyl sulfonyl fluoride buffer, pH 7.4) three times for 30 seconds using a Polytron at setting 8 (Brinkman). The homogenate was centrifuged 15 minutes at 2400 x g with no brake. The pellet (unbroken cells, nuclear material) was discarded, and the supernatant was centrifuged 20 minutes at 43 700 x g with no brake. The resulting supernatant was discarded. The upper white fluffy pellet (P1) was resuspended in sucrose-tris buffer. The lower dark pellet (P2) was resuspended in sucrose-tris buffer and centrifuged 20 minutes at 43700 x g with no brake. The supernatant was discarded and the upper white fluffy pellet resuspended in sucrose-tris buffer and combined with P1. The lower dark pellet was resuspended in sucrose-tris buffer and combined with the P2.

The combined P1 suspension was homogenized 15 seconds at setting 8, layered onto a 20% Percoll™ gradient, and centrifuged 30 minutes at 46 000 x g with maximum brake. The resulting white fluffy basolateral membrane layer was then centrifuged 30 minutes at 115 000 x g with maximum brake. The membrane layer was removed, resuspended in sucrose-tris buffer, and homogenized 15 seconds at setting 8. 1 *M* CaCl2 was added to a final concentration of 10 m*M* and the homogenate was stirred gently on ice for 10 minutes. The mixture was then centrifuged 10 minutes at 7700 x g. The supernatant was discarded and the pellet resuspended in sucrose-tris buffer. The resuspended pellet was then homogenized for 15 seconds at setting 8 and centrifuged 20 minutes at 46 000 x g. The supernatant was discarded and the final basolateral membrane pellet was resuspended in sucrose-tris buffer.

The combined P2 suspension was homogenized 15 seconds at setting 8 and centrifuged 10 minutes at 1900 x g. The resulting pellet was discarded and the supernatant was centrifuged 15 minutes at 14 600 x g. The pellet was discarded. 1 *M* $CaCl_2$ was added to the supernatant to a final concentration of 10 m*M* and the homogenate was gently stirred on ice for 20 minutes. The mixture was centrifuged 10 minutes at 3000 x g with maximum brake. The supernatant was then centrifuged 30 minutes at 46000 x g, and the resulting final brush border membrane pellet was resuspended in distilled deionized water. Membrane purity was confirmed through 10-20 fold enrichment of brush border and basolateral membrane markers, alkaline phosphatase and Na^+/K^+-ATPase, respectively. Aliquots were stored at -80°C.

Western Blot Analysis

Brush border and basolateral membrane proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel elecrophoresis using the Mini-Protean II electrophoresis system (BioRad Laboratories (Canada) Ltd., Mississauga, ON) and then transferred onto MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA). The blotting conditions were optimized by multiple trials to ensure complete protein transfer from gel to membrane. After blotting the membrane was stained with Amido Black to ensure homogenous protein transfer and the gel was stained with Commassie Blue to document complete protein transfer. Primary antibodies (Cedarlane Laboratories, Ltd., Hornby, ON) included anti-GLUT2 purified rabbit polyclonal IgG antibody (GLUT2 molecular weight, 60 kDa) and anti-SGLT-1 purified rabbit polyclonal IgG antibody (SGLT-1 molecular weight, 73 kDa). Immunoblotting was conducted according to the manufacturers instructions. Briefly, nonspecific binding sites were blocked by immersing the membrane in 5% nonfat dry milk in Tris-buffered saline with Tween 20 (TBST: composed of 20 mmol/L Tris, 137 mmol/L NaCl, 0.05% Tween 20, pH 7.6) for 1 hour at room temperature on a metabolic shaker. The membrane was then washed 3 x 10 minutes in TBST at room temperature. Primary antibodies were diluted 1:500 in TBST and incubated with membranes for 4 hours at room temperature. Blots were washed 3 x 10 minutes at room temperature and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG immunoglobulin (Pierce, Rockford, IL) at 1:2000 dilution in TBST for 1 hour at room temperature. Blots were washed again 3 x 10 minutes as described above. Positive fluorescence reaction was developed using the SuperSignal™

CL-HRP Substrate System (Pierce, Rockford, IL) and exposed to high performance luminescence detection Hyperfilm^{TM}-ECL (Amersham, Oakville, ON). Relative protein concentrations were determined using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) Ltd., Mississauga, ON).

Glucagon-Like Peptide-2 Radioimmunoassay

Blood was collected by cardiac puncture into a prechilled syringe containing EDTA (1 mg/mL blood, Sigma Chemical Co., St. Louis, MO) and aprotinin (500 KIU/mL blood, Sigma Chemical Co., St. Louis, MO). Plasma was separated by centrifugation and stored at -70°C for subsequent GLP-2 determination. Plasma GLP-2 concentration was quantified using a commercially available kit (Peninsula Laboratories, Inc., Belmont, CA). Briefly, peptides were extracted from plasma using C_{18} SEP-COLUMNS (Peninsula Laboratories, Inc., Belmont, CA), 0.1% trifluoroacetic acid and 60% acetonitrile as elution solvents (Peninsula Laboratories, Inc., Belmont, CA). Concentrations of GLP-2 were measured using a competitive ^{125}I-GLP-2 binding assay. This kit measures GLP-2 with 0% cross reactivity with the various GLP-1 forms. The IC_{50} for this assay is 108 pg/tube at a binding of 98.9% (defined as the mean total bound minus 2 SD from the mean total bound). The intra-assay coefficient of variance was 1.25%.

Statistical Analysis

All data are presented as mean±SEM. Statistical comparisons between treatments were determined using one-way analysis of variance (ANOVA). The northern and western blot results were analyzed using a randomized block ANOVA to account for variation due to multiple blots. When a significant difference was identified ($p<0.05$), comparisons of means were made using student's t-test. In addition, orthogonal polynomial regression was calculated to describe the curvilinear relationship between the variable and length of SCFA-supplemented TPN infusion. Computations were conducted using the general linear model procedure in SAS (Version 6.04, SAS Institute, Cary, NC).

RESULTS

Intestinal Protein, DNA and RNA Concentrations

Protein, DNA and RNA concentrations were measured in the jejunum and ileum to quantitate the extent of structural changes induced by acute periods of systemic SCFA administration. Jejunal protein concentration was not altered with SCFA supplementation, whereas, the ileal protein concentration was reduced ($p<0.003$) within 6 hours of supplementation (Table 8.1). The curvilinear relationship between ileal protein concentration and duration of SCFA infusion was best described by a cubic polynomial ($y=b_o + b_1x + b_2x^2 + b_3x^3$; $p=0.0005$). The DNA concentration did not differ between groups indicating that SCFA administration did not acutely affect DNA concentration in the jejunum or ileum. However, the concentration of DNA was significantly higher ($p<0.05$) in the ileum than the jejunum in both the 12 and 72 hour SCFA groups (Table 8.1). The RNA concentration did not differ between groups in the jejunum or ileum (Table 8.1).

GLUT2 mRNA Abundance

Jejunal GLUT2 expression was increased ($p=0.0001$) in the SCFA groups at all time points compared to the control TPN group (Figure 8.2). Within the SCFA groups, jejunal GLUT2 expression peaked following 6 and 12 hours of SCFA administration, and then slowly fell to a significantly lower level in the 72 hour SCFA group. The curvilinear relationship between GLUT2 mRNA abundance and duration of SCFA infusion in the jejunum can be described by a cubic polynomial ($y=b_o + b_1x + b_2x^2 + b_3x^3$; $p=0.0004$). GLUT2 mRNA abundance was barely detectable in total cellular RNA from the ileum of all groups and as such this data will not be reported.

GLUT2 and SGLT-1 Protein Abundance

The abundance of GLUT2 and SGLT-1 protein was measured in the jejunum and ileum to determine the amount of transporter protein within the basolateral and brush border membrane, respectively. In the jejunal basolateral membrane, GLUT2 protein abundance tended to increase (p=0.1) following 6, 12 and 24 hours of SCFA supplementation (Table 8.2). In the ileum, GLUT2 protein abundance increased ($p<0.05$) in the 6, 12 and 24 hour SCFA-supplemented groups when compared to the control TPN group (Table 8.2). The 72 hour SCFA-supplemented group did not differ from the other SCFA supplemented groups, however it was not significantly higher than the control TPN group. The curvilinear relationship between ileal GLUT2 protein abundance and duration of SCFA infusion can be described by a cubic polynomial ($y=b_o + b_1x + b_2x^2 + b_3x^3$; p=0.001). Jejunal and ileal SGLT-1 protein abundances in the isolated brush border membrane were not influenced by SCFA-supplemented TPN at any time point (Table 8.2).

Proglucagon mRNA Abundance

Ileal proglucagon expression was upregulated ($p<0.003$) following 6, 12 and 24 hours of SCFA-supplemented TPN, but returned to the control TPN level following 72 hours of SCFA administration (Figure 8.3). The curvilinear relationship between ileal proglucagon mRNA abundance and duration of SCFA infusion can be described by a quadratic polynomial ($y=b_o + b_1x + b_2x^2$; p=0.0003). Systemic SCFA administration had no effect on proglucagon mRNA abundance in the colon (Figure 3). Furthermore, the abundance of proglucagon mRNA in the colon was significantly less ($p<0.02$) than in the ileum of the 6 hour SCFA supplemented group (Table 8.2).

Plasma Glucagon-Like Peptide-2 Concentration

To examine if the increased proglucagon mRNA abundance in the ileum was translated into the encoded peptides, GLP-2 concentrations were measured in the plasma. Plasma GLP-2 concentrations were higher ($p<0.03$) following 12 hours of SCFA-supplemented TPN when compared to the TPN control, 24 and 72 hour SCFA-supplemented groups. The 6 hour SCFA group was not significantly lower than the elevated 12 hour SCFA group, however it did not differ from all other groups as well (Figure 8.4). The curvilinear relationship between plasma GLP-2 concentration and duration of SCFA infusion can also be described by a cubic polynomial ($y=b_o + b_1x + b_2x^2 + b_3x^3$; p=0.004).

c-myc, c-fos and c-jun mRNA Abundance

In jejunal total cellular RNA, c-*myc* expression was significantly higher ($p=0.004$) following 6, 12 and 24 hours of SCFA supplementation but returned to control TPN levels in the 72 hour SCFA group (Figure 8.5). A similar response was found with c-*myc* expression in the ileum, however the 12 hour SCFA group was not significantly higher than the 72 hour SCFA or control TPN groups (Figure 8.5). The 6 and 24 hour SCFA groups were both higher ($p<0.02$) than the 72 hour SCFA and control TPN group. The c-*myc* expression in the colon did not differ between groups (Figure 8.5). The drop in c-*myc* expression in the ileum of the SCFA-12 group resulted in the quadratic polynomial relationship ($y=b_o + b_1x + b_2x^2$; $p<0.01$) between c-*myc* and duration of SCFA infusion, whereas a cubic polynomial relationship ($y=b_o + b_1x + b_2x^2 + b_3x^3$; $p<0.05$) was found in the jejunum.

The c-*jun* mRNA abundance in total cellular RNA did not differ between groups in the jejunum and the colon. c-*jun* expression in the ileum peaked ($p<0.05$) following 12 hours of SCFA supplementation and gradually returned to control levels following 72 hours of SCFA supplementation (Figure 8.6). This curve can be described by a quadratic curvilinear relationship ($y=b_o + b_1x + b_2x^2$; $p<0.05$). The abundance of c-*jun* mRNA was higher ($p<0.05$) in the colon than the ileum of the 6 and 24 hour SCFA groups, and in the jejunum in the 12, 24 and 72 hour SCFA groups (Figure 8.6).

In jejunal and colonic total cellular RNA, c-*fos* expression did not differ between groups (Figure 8.7). Ileal c-*fos* expression increased ($p=0.0001$) following 6, 12 and 24 hours of SCFA supplemented TPN and returned to control TPN levels in the 72 hour SCFA group (Figure 8.7). This curve can be described by a quadratic curvilinear relationship ($y=b_o + b_1x + b_2x^2$; $p=0.0001$). The abundance of c-*fos* mRNA was higher ($p<0.03$) in the colon than in both the jejunum and ileum of the 12 hour SCFA group.

DISCUSSION

There is a growing body of literature suggesting that systemic SCFAs prevent TPN-induced atrophy in normal (Koruda et al., 1990) and resected small intestine (Koruda et al., 1988; Chapters 2 and 3). However, little is known about the early events following administration and the mechanism regulating this phenomena. This study uses a TPN model to examine acute systemic SCFA administration in a rapid time course fashion. An advantage of this model is that it is independent of the timing and amount of nutrients being delivered to the distal gastrointestinal tract.

Traditionally, intestinal adaptation has been quantified by measuring structural markers of the intestinal mucosa one week after intestinal injury. In this study, systemic SCFA supplementation for less than 72 hours had no effect on DNA and RNA concentration in the ileum or jejunum, whereas ileal protein concentration was unexpectedly lower in the SCFA groups at all time points. The short administration periods used may not have allowed enough time to produce the changes in DNA and RNA concentrations observed in previous studies (Koruda et al., 1988, 1990; Chapter 2). The lower protein concentration within the SCFA groups may support the hypothesis that an increased proportion of cells are undergoing mitosis and may be functionally immature (Menge et al., 1982). In the current study, ileal DNA and RNA concentrations would be elevated if expressed per unit protein, but results have been expressed per mg tissue weight to be consistent with our previous study (Chapter 2). Thus, it appears that structural changes observed following 1 week of SCFA supplementation cannot be measured within 72 hours of SCFA administration in rats with normal, unresected small intestine.

GLUT2 gene expression is acutely upregulated with systemic SCFAs and western analysis confirms more abundant GLUT2 protein in the ileum and trends toward increased protein in the jejunum. This correlates with increased GLUT2 mRNA and functional glucose uptake in the ileum of rats having undergone an 80% proximal resection (Chapter 3). The more modest change in ileal GLUT2 mRNA in this study versus the resection study reinforces the concept that the ileum adapts to compensate for the resected portion following a proximal resection. To our knowledge, this is the first study assessing the functional response to SCFA-supplemented TPN in the normal, unresected small intestine.

The SGLT-1 transporter protein abundance in the brush border membrane was not altered by SCFA-supplemented TPN. This may not be surprising since these animals received all nutrients parenterally. We have previously reported increased functional glucose uptake in the residual ileum of animals receiving SCFA-supplemented TPN (Chapter 3) which suggested increased SGLT-1 functional capacity. Additional studies are needed to determine if the trophic force of intestinal resection prompts a SGLT-1 response or if systemic SCFAs differentially affect brush border and basolateral glucose transporters.

It is of interest, that differential effects were noted in the ileum and colon with regard to proglucagon gene expression. The ileum seems to be particularly responsive to systemic administration of SCFA. In addition to increased c-*myc*, c-*jun* and c-*fos* expression in the ileum, the GLUT2 transporter protein is upregulated in the ileum. This ileal specific response may be due to a local effect of L-cells neighbouring ileal enterocytes or increased sensitivity to plasma GLP-2. Temporal changes in plasma GLP-2 concentration and GLUT2 expression and protein abundance can be described by a cubic polynomial lending support to a recent report that GLP-2 upregulates basolateral membrane glucose transport (Cheeseman and Tsang, 1996).

The drop in plasma GLP-2 levels to control levels following 72 hours of SCFA supplementation corresponds with the curve seen for proglucagon mRNA abundance. Mathematical modelling indicated that proglucagon expression and GLP-2 concentrations peak within the first 24 hours of SCFA administration. This corresponds with the marked increase in proglucagon mRNA levels following massive small bowel resection reported by Taylor and associates (Taylor et al., 1990). As massive small bowel resection is known to acutely upregulate proglucagon mRNA abundance, it would be useful to assess the effect of SCFA-supplemented TPN in the early post-surgery period.

Multiple factors are purportedly important in the mediation of intestinal proliferation. However, it is the earliest cellular responses which are believed to be the proliferative stimulus and are associated with *in vivo* intestinal growth. Following intestinal resection in rats, Sacks and associates (Sacks et al., 1995) reported a marked induction of c-*myc* expression 1 - 3 hours following resection in the absence of nutrient intake. These authors suggests that changes in c-*myc* expression precedes the rise in proglucagon mRNA. In the present study, c-*myc* and proglucagon mRNA are upregulated within 6 hours of supplementation and both exhibit a quadratic curvilinear response for the duration of supplementation, suggesting further study is necessary to establish signaling response priorities.

Recently, Velázquez and associates (1996) reported that 24-hour *in vivo* exposure of normal rat colonic mucosa to butyrate induced c-*Jun* but not c-*Fos* protein abundance. Similarly, we did not observe changes in c-*fos* mRNA abundance, but found a trend (p=0.1) towards increased expression of c-*jun* in the colon of groups receiving SCFA infusions for 24 hours or less. We believe the reduced effect of butyrate on colonic c-*jun* and c-*fos* expression in the present study may be a result of systemic versus "topical" administration of butyrate and/or the amount applied. The data reported herein leads us to believe that the ileum is highly sensitive to systemic SCFAs, but segmental responses may differ with mode of nutrient delivery. Although not altered by systemic SCFAs, the importance of transcriptional regulators in the colon should not be overlooked as the expression of both c-*jun* and c-*fos* was higher in the colon than the ileum at various time points.

In summary, systemic SCFAs produce a similar pattern in plasma GLP-2 concentrations and ileal GLUT2 expression and protein abundance. Similarly, ileal c-*myc* and proglucagon expression respond uniformly over time to systemic SCFAs suggesting a cooperative role for these genes and proteins during intestinal proliferation. Although SCFA supplementation seems to modulate adaptation of the entire gastrointestinal tract, the ileum seems to be most responsive. Additional experiments are underway to elucidate signals potentiating intestinal adaptation.

Table 8.1

Effect of Various Lengths of SCFA- Supplemented TPN on Jejunal and Ileal Protein, DNA and RNA Concentrations.[1,2]

	TPN-72 (n=7)	**SCFA-6** (n=8)	**SCFA-12** (n=7)	**SCFA-24** (n=8)	**SCFA-72** (n=8)	**p value**
jejunal protein (μg pro/mg tissue)	103.0 ± 5.4	95.2 ± 5.4	92.4 ± 5.0	90.2 ± 4.7	98.2 ± 5.9	NS
ileal protein	113.0 ± 5.2^a	87.1 ± 4.8^b	91.1 ± 4.8^b	83.0 ± 4.8^b	95.9 ± 4.8^b	$p = 0.002^3$
jejunal DNA (μg DNA/mg tissue)	3.5 ± 0.6	3.2 ± 0.5	2.7 ± 0.5*	4.1 ± 0.5	3.5 ± 0.5**	NS
ileal DNA	5.6 ± 1.1	4.9 ± 1.0	5.4 ± 1.0*	5.3 ± 0.9	7.0 ± 1.0**	NS
jejunal RNA (μg RNA/mg tissue)	3.5 ± 0.3	3.2 ± 0.3	2.9 ± 0.3	3.0 ± 0.3	3.5 ± 0.3	NS
ileal RNA	3.4 ± 0.4	3.2 ± 0.4	2.7 ± 0.4	2.6 ± 0.4	3.9 ± 0.4	$p < 0.1$

[1] Values are means ± SEM.

[2] Means within a row with different superscripts are significantly different ($p<0.05$).

[3] The relationship between the variable within this row and length of TPN+SCFA infusion can be described by a cubic polynomial.

* Indicates a significant difference between gastrointestinal segments containing (*) within that coloumn ($p<0.02$).

** Indicates a significant difference between gastrointestinal segments containing (**) within that coloumn ($p=0.001$).

Table 8.2

Effect of Various Lengths of SCFA- Supplemented TPN on GLUT2 and SGLT-1 Protein Abundance[1]

densitometry units	TPN-72 (n=4)	SCFA-6 (n=4)	SCFA-12 (n=4)	SCFA-24 (n=4)	SCFA-72 (n=4)	p value
jejunal GLUT2	1.2 ± 0.8	2.5 ± 0.8	4.1 ± 0.8	4.0 ± 0.8	2.0 ± 0.8	p = 0.1
ileal GLUT2	0.5 ± 2.0^a	9.2 ± 1.9^b	8.4 ± 2.0^b	6.7 ± 2.0^b	5.2 ± 2.0^{ab}	$p < 0.05$[2]
jejunal SGLT-1	0.3 ± 1.0	1.8 ± 1.0	1.2 ± 1.0	3.3 ± 1.0	2.9 ± 1.0	NS
ileal SGLT-1	2.8 ± 0.6	3.6 ± 0.6	2.5 ± 0.8	4.0 ± 0.6	3.0 ± 0.6	NS

[1] Values are means ± SEM.

[2] The relationship between the variable within this row and length of TPN+SCFA infusion can be described by a cubic polynomial.

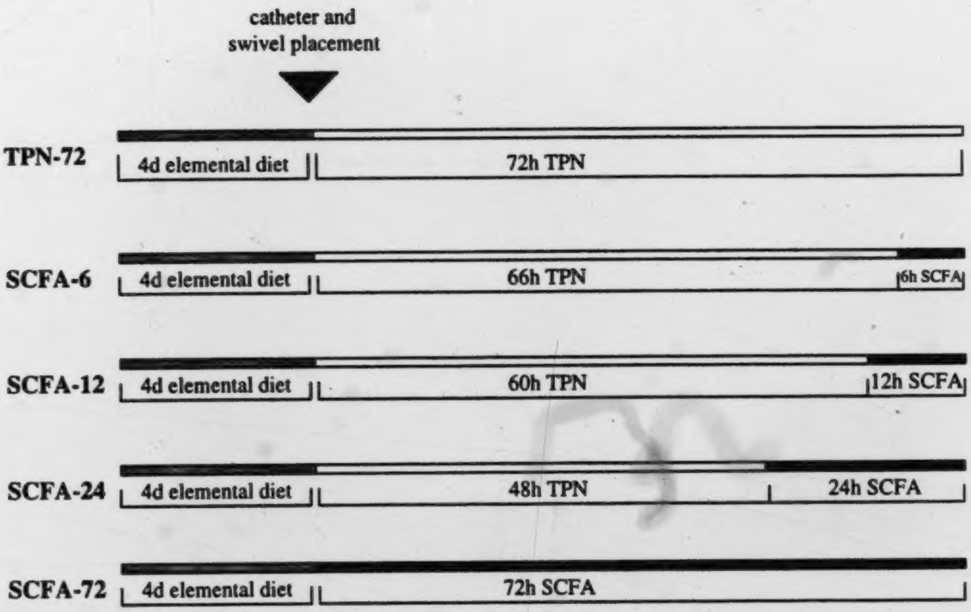
catheter and
swivel placement
TPN-72
4d elemental diet
72h TPN
SCFA-6
4d elemental diet
66h TPN
6h SCFA
SCFA-12
4d elemental diet
60h TPN
12h SCFA
SCFA-24
4d elemental diet
48h TPN
24h SCFA
SCFA-72
4d elemental diet
72h SCFA

Figure 8.1 Experimental Design.

Following a 4-day acclimatization period animals underwent a jugular catheterization and swivel placement. All animals received 72 hours of continuous nutrient infusion as either standard total parenteral nutrition (TPN) or an isoenergetic, isonitrogenous formulation containing short-chain fatty acids (SCFAs). Animals were randomly assigned to 1 of 5 treatments: standard TPN for 72 hours (TPN-72); SCFA-supplemented TPN for 72 hours (SCFA-72); or standard TPN followed by SCFA-supplemented TPN for the final 6 (SCFA-6), 12 (SCFA-12) or 24 (SCFA-24) experimental hours.

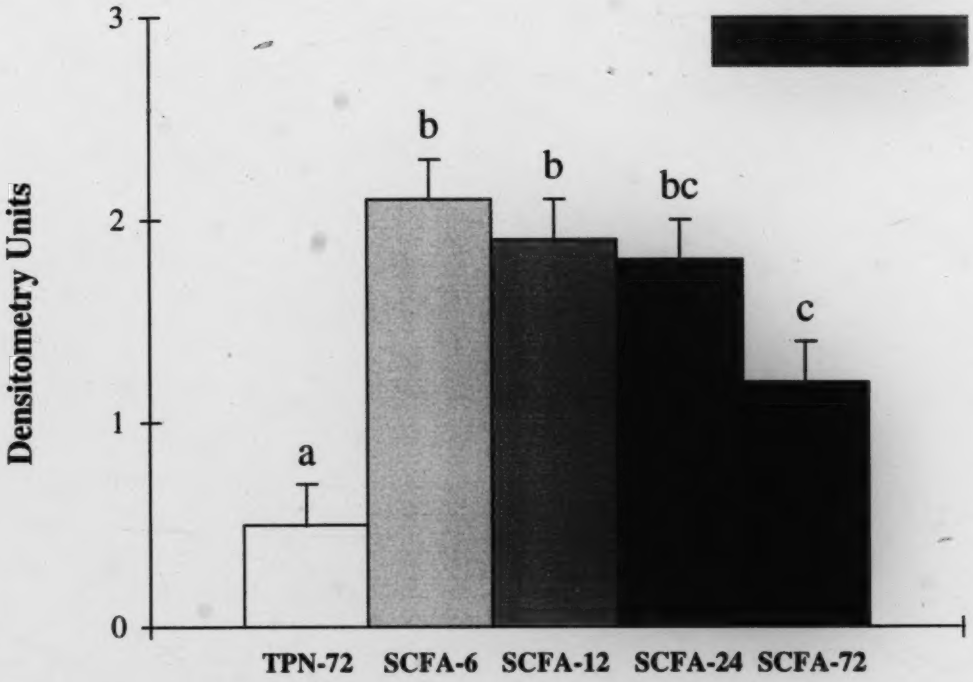

Figure 8.2 Modulation of Jejunal GLUT2 mRNA Abundance by SCFA-Supplemented TPN.

Data are means±SEM. Jejunal GLUT2 expression was increased (P=0.0001) in the SCFA-supplemented TPN groups at all time points compared to the control TPN-72 group. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

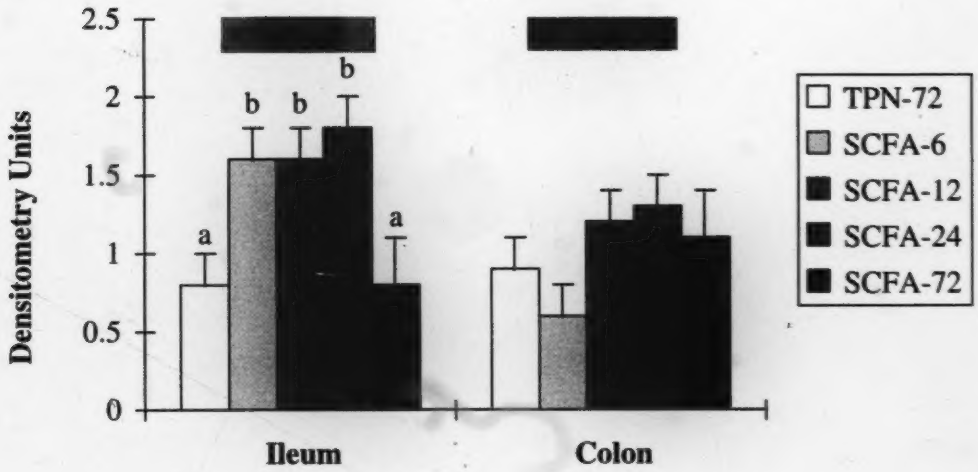

Figure 8.3 The Effect of SCFA-Supplemented TPN on Ileal and Colonic Proglucagon mRNA Abundance.

Data are means±SEM. Ileal proglucagon expression was upregulated ($p<0.003$) following 6, 12 and 24 hours of SCFA-supplemented TPN, but returned to control TPN level following 72 hours of SCFA administration. SCFA-supplemented TPN had no effect on colonic proglucagon mRNA abundance at any time point. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

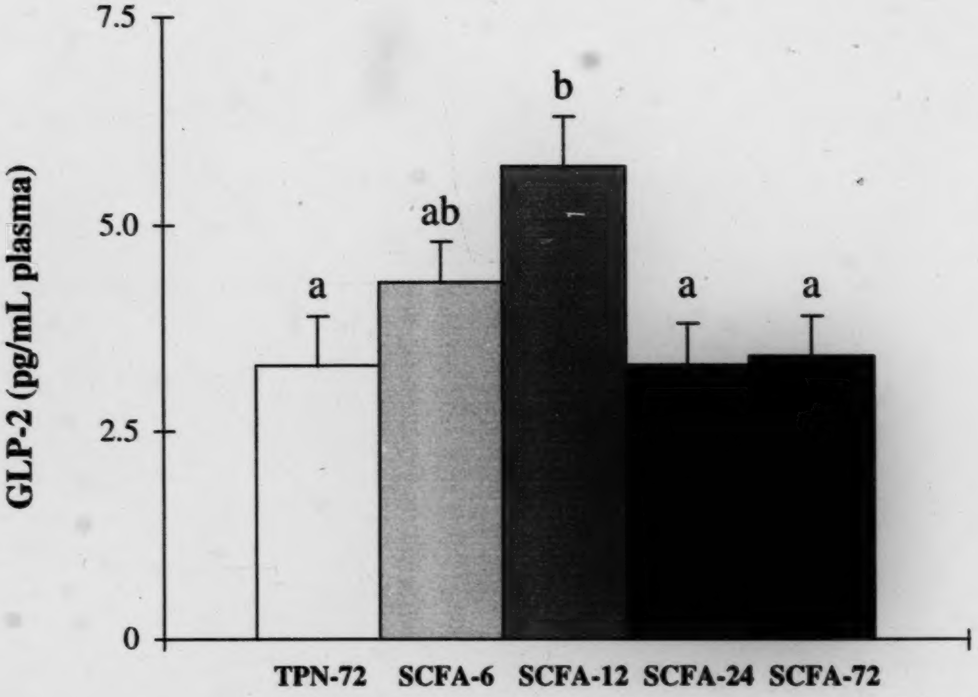

Figure 8.4 Modulation of Plasma GLP-2 Concentration by SCFA-Supplemented TPN.

Data are means±SEM. Plasma GLP-2 concentration were higher ($p<0.03$) following 12 hours of SCFA-supplemented TPN when compared to the TPN-72, SCFA-24 and SCFA-72 groups. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

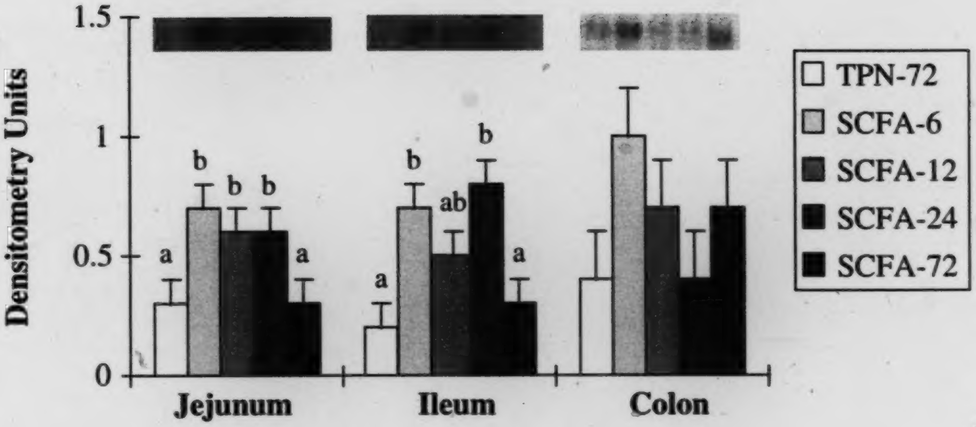

Figure 8.5 The Effect of SCFA-Supplemented TPN on c-*myc* mRNA Abundance in the Gastrointestinal Tract.

Data are means±SEM. Jejunal c-*myc* expression was higher ($p=0.004$) following 6, 12 and 24 hours of SCFA supplementation but returned to TPN-72 levels in the SCFA-72 groups. In the ileum, there was a similar response to SCFA-supplemented TPN, however the SCFA-12 group was not higher than the TPN-72 or SCFA-72 groups. No differences were found in the colon. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

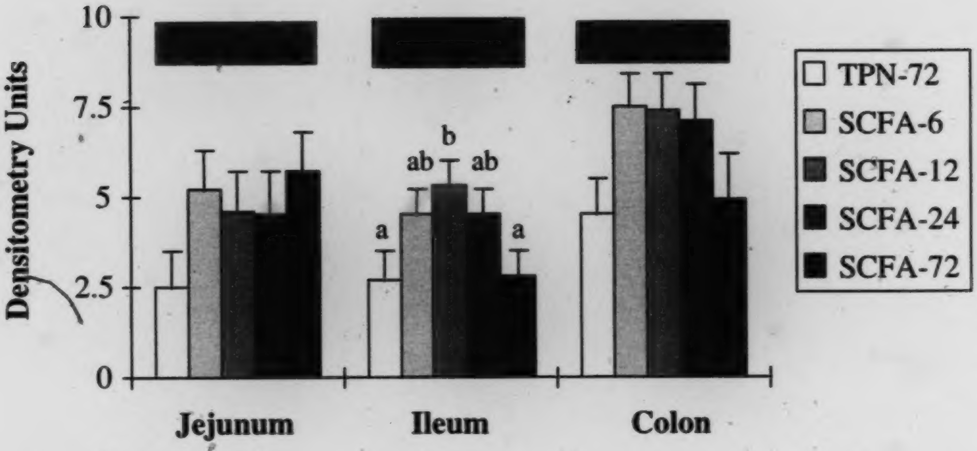

Figure 8.6 Modulation of Gastrointestinal c-*jun* mRNA Abundance by SCFA-Supplemented TPN.

Data are means±SEM. c-*jun* mRNA abundance did not differ between groups in the jejunum and colon. In the ileum, c-*jun* expression peaked ($p<0.05$) following 12 hours of SCFA supplementation and gradually returned to control levels following 72 hours of SCFA-supplementation. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

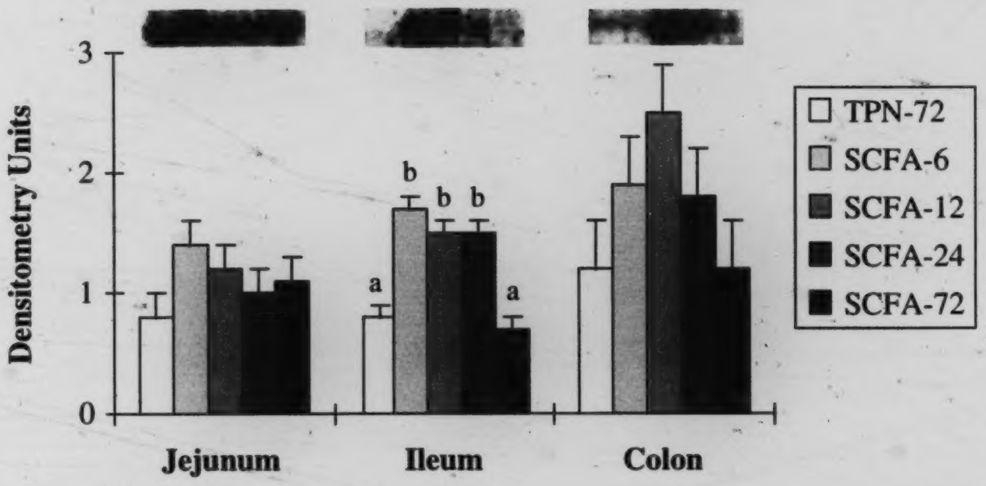

Figure 8.7 The Effect of SCFA-Supplemented TPN on c-*fos* mRNA Abundance in the Gastrointestinal Tract.

Data are means±SEM. Ileal c-*fos* expression increased (p=0.0001) following 6, 12 and 24 hours of SCFA-supplemented TPN and returned to control TPN levels in the 72 hours SCFA group. Jejunal and colonic c-*fos* expression did not differ between groups. Bars with different letters are significantly different as identified by two-way ANOVA and student's t-test.

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CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

Intestinal adaptation is complex physiological process that is not completely understood. Following intestinal resection, the remnant gut undergoes both structural and functional modifications in an apparent attempt to compensate for reductions in absorptive capacity. Although this response has been widely described, the molecular and cellular mechanisms regulating this phenomena are largely unknown. Nutrition is known to be a central factor in this process as both specific nutrients and route of administration influence the enteroplastic response. Similarly, there is a substantial body of research implicating various systemic factors as enterotrophic signals. My goal was to examine the effect of SCFAs on enterotrophic factors and consequent interorgan responses in normal and resected animals.

EXPERIMENTAL STRATEGIES

The objectives of this thesis research were:

1) to identify mechanism(s) by which SCFAs are trophic to the gastrointestinal tract;
2) to provide insight into regulatory mechanisms of intestinal adaptation, in both resected and normal nonresected intestine, and;
3) to determine if SCFA supplementation of TPN influences the multiorgan response to surgical trauma.

Several studies were conducted to attain these goals and a brief rationale for some of the strategies employed is provided.

80% Proximal Intestinal Resection

Although many other models of intestinal adaptation exist (i.e. diabetes, starvation/refeeding, pregnancy, lactation, etc.), intestinal resection is the most widely used. This model reliably produces a syndrome characterized by massive prolonged diarrhea, malabsorption of enteral nutrients, negative nitrogen balance and loss of body weight. The intensity of the adaptive response is proportional to the amount of intestine removed (Hanson et al., 1977), thus an 80% resection was selected to amplify the associated responses. As the magnitude of the adaptive response is greater after proximal as opposed to distal resections (Williamson, 1978) and the maintenance of the ileum preserves the distally located L-cells from which the potentially trophic proglucagon-derived peptides are secreted (Evans and Potten, 1988), we used a proximal intestinal resection in these studies.

Total Parenteral Nutrition

TPN provides an ideal model to study the role of specific nutrients in intestinal adaptation following resection. The use of oral diets following resection results in malabsorption and malnutrition thus confounding nutrient effects with those of under-nutrition and lower body weight. Thus, TPN allows examination of distinct nutrients while enabling the provision of adequate nutrition support. In addition, the systemic provision of nutrients eliminates direct contact of intraluminal nutrients with the epithelial cells and subsequent secretion of trophic upper gastrointestinal secretion. Continuous nutrient infusions allows one to examine patterns of gene expression independent of time of last meal consumed which may reduce diurnal variations in mRNA and protein abundance. Finally, the ability of systemically administered nutrients to prevent TPN associated mucosal atrophy thus enhancing intestinal adaptation is of significant clinical relevance.

Measurement of Specific Messenger RNA

Northern blot analysis was a technique used extensively within this series of experiments. A key technique in molecular biology, its principal aim is the measurement of a specific mRNA. Information yielded by this technique is useful for two main reasons. First, the measurement of mRNA allows investigators to determine which tissues express a particular gene and thus indicates the physiological function of the encoded protein. Secondly, and more pertinent to these studies, the measurement of mRNA determines the factors which regulate the expression of a given gene, be they nutritional, hormonal, or environmental. Although the level of regulation and post-translational processing are important considerations with all genes under investigation, northern blot analysis permits examination of factors which regulate the first step in protein production - gene expression.

SHORT CHAIN FATTY ACIDS INFLUENCE THE MULTIORGAN RESPONSE TO INTESTINAL RESECTION

Hypothesis 1: SCFA-supplemented TPN enhances structural and functional aspects of intestinal adaptation following intestinal resection, and is associated with immunoenhancement and reduced skeletal muscle catabolism.

SCFA-supplemented TPN following MSBR enhanced structural and functional markers of intestinal adaptation (increased ileal protein, DNA and RNA concentration, higher *in vitro* glucose uptake and GLUT2 mRNA abundance), improved various aspects of non-specific immunity (prevented abnormal hematological profiles, enhanced NK cell cytotoxicity) and halted skeletal muscle wasting (reduced muscle atrophy, decreased *in vitro* degradation rate, reduced urine 3-methylhistidine excretion, increased plasma glutamine concentration). These studies suggest that it is possible to add nutrients (i.e. SCFAs) to TPN which mimic the use of enteral solutions and enhance the multiorgan response to MSBR. These studies are important because they reflect an integrated investigative approach which is sometimes lacking despite the use of physiologically relevant *in vivo* models.

The mechanism by which SCFA-supplemented TPN enhance the multiorgan response is not elucidated in the current studies. SCFAs do not appear to directly modulate NK cell cytotoxicity and skeletal muscle protein turnover *in vitro* (Chapter 6) in tissue from rats with nonresected, intact small intestine, suggesting the involvement of secondary factors.

However, SCFAs may decrease bacterial translocation and thus, reduce immune activation to combat the mounting sepsis by enhancing gastrointestinal structure. The elimination of this potential immune response would reduce oxidative metabolism by immune cells as well as the release of sepsis-related mediators (i.e. IL-1 and TNF-α) both of which would aggravate skeletal muscle protein catabolism. Studies aimed at determining the effect of systemic SCFA supplementation on bacterial translocation and cytokine production would help substantiate this hypothesis.

Alternatively, the gastrointestinal tract may be directly mediating skeletal muscle and immune systems in a more dynamic manner. Following MSBR, SCFA-supplemented TPN increased the expression of IL-1β in the remnant ileum. This increase may represent a signal by cells of the gastrointestinal tract to the immune system of changes in mucosal barrier function and pending bacterial translocation. The lack of substrate provided to the gastrointestinal tract by standard parenteral solutions may compromise gastrointestinal function so that potential pathogens pass unrecognized or unmet metabolic demands prevent the signaling of immune effector cells. A possible consequent within this inadequate mucosal defense scenario is a systemic immune response and skeletal muscle catabolism. Future investigations should focus on SCFAs effect on mucosal immunity and the recruitment of systemic immune cells.

Finally, SCFAs may be exerting multiorgan effects *in vivo* because they are a preferential fuel for cells of the gastrointestinal tract. The provision of SCFAs as an alternative gastrointestinal fuel may reduce the demand for serosally-supplied glutamine and provide immune cells with a more steady state of this conditionally essential nutrient. The conservative amounts of SCFAs administered in the previous studies leads us to believe that the effects of SCFAs are due to their ability to alter interorgan signaling mechanisms, however studies aimed at elucidating these effects are needed.

SHORT-CHAIN FATTY ACIDS INDUCE ENTEROPLASTIC CHANGES AND UPREGULATE POTENTIAL GUT GROWTH FACTORS

Hypothesis 2: Systemic SCFAs rapidly modulate the gene expression of factors believed to be essential in regulation of intestinal adaptation.

SCFAs increased proglucagon and ornithine decarboxylase gene expression and resulted in various structural and functional adaptations at the cellular level. These alterations were further characterized in the unresected normal rat intestine and found to be associated with upregulation of proglucagon, c-*myc*, c-*jun* and c-*fos* mRNA abundance.

Changes in ileal protein concentration did not increase as quickly as ileal DNA and RNA concentration in response to SCFAs following intestinal resection. In the normal unresected intestine, protein concentration was rapidly lowered (<6 hours) with SCFA supplementation. The lower protein concentration within the SCFA groups may support the hypothesis than an increased proportion of cells are undergoing mitosis and may be functionally immature (Menge et al., 1982). The decreased Na^+, K^+-ATPase mRNA following SCFA-supplemented TPN may be another example of immature cells as activity of this basolateral protein is reduced in the less differentiated cells of the crypt (Madsen et al., 1992). However, as this structural indices is increased following 7 days of SCFA-supplemented TPN in the resection model, it appears that the lower concentration seen with acute SCFA supplementation may provide a further example of the dissociation between structural and functional aspects of intestinal adaptation.

SCFAs rapidly (<6 hours) upregulate the expression of GLUT2 mRNA suggesting that SCFAs increase basolateral hexose transport in the normal and resected intestine. This is the first report that a specific nutrient (i.e. SCFA) may modulate gene expression of other nutrient transporters (i.e. glucose). These TPN studies also support Cheeseman's hypothesis (1992) that the primary locus for any adaptive or regulatory response in nutrient uptake is at the basolateral membrane. Future studies using *in situ* hybridization would identify if GLUT2 transporter capacity is determined only in the crypt cells as with substrate-induced nutrient regulation (Ferraris and Diamond, 1993) or if cells migrating along the crypt-villus axis can be reprogrammed to increased basolateral glucose transport in response to SCFAs.

It remains unclear how systemic SCFAs modulate basolateral membrane transport. Butyrate has been reported to be a cofactor of regulatory transcription proteins in the nucleus which are directly involved in gene expression (Kruh et al., 1995) and to interact with G-proteins, key components of the signal transduction pathway in cellular growth and differentiation (for review, see Válezquez et al., 1996). It is not known if SCFAs act on all cell types or preferentially on enteroendocrine cells.

Proglucagon gene expression is increased with SCFA following MSBR and in the normal unresected ileum. Enteroplasticity has been associated with increased synthesis of intestinal proglucagon and secretion of proglucagon-derived peptides. Similarly, ileal c-myc and proglucagon expression respond uniformly over time to systemic SCFAs suggesting a cooperative role for these genes and proteins during intestinal adaptation. The rapid responses observed in the TPN model make it a suitable candidate to apply antagonists to investigate the relative importance of proglucagon-derived peptides versus early response genes in modulating intestinal adaptation.

In conclusion, these studies begin to elucidate underlying molecular mechanisms whereby known gastrointestinal specific nutrients (i.e. SCFAs) can be added to TPN to mediate intestinal adaptation. Insight is provided into the sequence of signaling events involving gastrointestinal growth factors, polyamine synthesis and nutrient transporters. Finally, the relative role of the gastrointestinal epithelium and distant organs such as skeletal muscle and peripheral immunity is explored. This information will enable health professionals to devise new strategies to manipulate the intestine and maximize nutrient utilization in the absence of luminal substrate.

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